The Effect of GABAergic Neuron Ablation on Seizure Frequency and Sleep Cycle Disruption in Mice

Authored by: Haley Folsom

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Research Information

Faculty Mentor: *Dr. Robert Gross*

Second Reader: *Dr. Mary Holder*

Additional Researchers :

Claire Anne Gutenkunst, PhD, Assistant Professor
Emory University Department of Neurosurgery

Alejandra Fernandez, PhD, Post Doctoral Fellow
Emory University Department of Neurosurgery

Location of Research:

Translational Neuro-Engineering Research Laboratory at Emory University - Dr. Robert E. Gross, MD, PhD
Woodruff Memorial Research Building, Atlanta, GA 3032

Abstract

Epilepsy is a common neurological condition, prevalent in about 3.8% of people in the U.S. and increasing exponentially, making it a key area of research (England et al, 2012). The primary goal of this research study is to analyze REM sleep cycle disruptions due to seizures resulting from GABAergic neuron ablation within CASpase (CAS) mouse models. This ablation is done by using two unilateral injections of AAV to induce cell death of GABAergic neurons, which are primary inhibitory neurons essential for suppressing hypersynchronous neural activity that underlies seizure activity. The results of this study are extremely important because sleep deprivation is known to be a trigger for spontaneous seizure activity and could be a contributor to worsening seizures in those patients with epilepsy. The results of this study can be used to find multifaceted treatments for both sleep disorders and seizures activity.

Keywords: temporal lobe epilepsy, caspase, kainic acid, REM sleep, sleep cycle, seizure, EEG

Introduction

Epilepsy is a neurological disorder, categorized by a surge of abnormal brain activity causing seizures, changes in behavior, and alterations to an individual's senses (Thijs et al, 2019). According to the World Health Organization (WHO), this disorder is relatively common, affecting approximately 70 million people worldwide (Thijs et al, 2019). Epilepsy appears in various forms and impacts each patient differently, so it is very difficult for researchers to pinpoint its exact origin. In fact, in roughly 50% of cases the cause of epilepsy is unknown (University of Chicago Medicine, 2019). In addition, there is a wide range of epileptic symptoms from abnormal behaviors to sleep disruptions to changes in eating habits, which have been a focal point of recent research (Zhao et al, 2014).

Sleep analysis is important to consider when studying epilepsy because it has been estimated that 40% of those with epilepsy have experienced seizure activity during sleep (Manni & Terzaghi, 2010). Furthermore, research has shown that those with focal epilepsies are twice as likely to experience sleep disturbance in comparison to controls (Bazil, 2003). In addition, researchers have been able to pinpoint that different stages throughout the sleep cycle contribute to seizure onset differently, with non-Rapid Eye Movement (nREM) stages elevating and Rapid Eye Movement (REM) suppressing seizure activity (Manni & Terzaghi, 2010). This is critical because sleep disruptions often yield less time spent in REM sleep, which has been demonstrated to suppress seizures (Kunz et al, 2004). This sparked inquiry into whether seizure onset can be determined via preceding changes in sleep cycles, thus inspiring the basis for this project.

This research aims to further explore the pre-established correlation between epilepsy and sleep disturbances. Although there have been studies observing a correlation between epilepsy and sleep, there has yet to be a clear understanding of the connection. One of the most frequently used animal models of temporal lobe epilepsy (TLE), a common form of focal epilepsy, is the kainic acid (KA) model (Epilepsy Foundation, 2019). This KA model will serve as a comparison tool against the CAS model, which uses unilateral adeno-associated virus (AAV) to ablate specific GABAergic neurons and induce seizures. The model is coupled with "Pedersen-custom-made (Zhu et al, 2020)" head caps, which monitor brain signals by electroencephalography (EEG) and capture seizure and sleep activity. This set-up allows for comparison of sleep patterns and disturbances before (control state) and after administration of KA or AAV (seizure/epilepsy state), to determine if sleep disruptions precede seizure activity.

Literature Review

Epilepsy is a neurological disorder in which brain activity becomes abnormal, often causing seizures associated with periods of unusual behavior, sensations, and sometimes loss of awareness. While it was first documented back in 460 BC (Mayo Clinic, 2019), it was not until the early 2000s that researchers started gaining more insight into the disease mechanism and its effect on people's lives. According to the Center for Disease Control (CDC, 2019), it is estimated that approximately 3 million people in the United States are currently living with epilepsy, making it one of the most common neurological disorders.

Foundation of Epilepsy:

Epilepsy has been defined by the World Health Organization (WHO) as, "a common medical disorder resulting in a tendency to produce recurrent seizures" (World Health Organization, 2019). Considering frequent seizure activity is a fundamental component in the conditions of epilepsy, it begs the question as to what specifically constitutes a seizure. At this point, the most commonly accepted definition is that a seizure is characterized by a rapid increase in the frequency and amplitude of neural signaling as measured by EEG recording devices. There are currently more than thirty different types of seizures that have been identified and recognized by the CDC, each falling into one of three categories: idiopathic, symptomatic, or cryptogenic epilepsy. Idiopathic generalized epilepsy has been classified by a lack of structural brain lesion evidence shown via MRI, as well as minimal symptoms and signs in periods between seizure activity (McWilliam & Al Khalili, 2019). Symptomatic epilepsy is the result of a bodily injury, often resulting from traumatic brain injuries (TBI), strokes, infections, and other similar trauma. Seizure activity that cannot be classified as idiopathic or symptomatic epilepsy is denoted as cryptogenic, due to the seizure's unknown causation.

Characteristics of Idiopathic Epilepsy. Idiopathic epilepsies account for approximately one-third of all epilepsies and tend to have an underlying genetic causation (Panayiotopoulos, 2008). This specific seizure class is categorized by typical absences, myoclonic jerks, and generalized tonic-clonic seizures, alone or in varying combinations and severity (Panayiotopoulos, 2008). There is not one specific cause of idiopathic epilepsy, even though inheritance genetics and random mutations are thought to be major contributors.

Causation of Symptomatic Epilepsy. The definition of symptomatic seizures has been denoted as, "a clinical seizure occurring at the time of a systemic insult or in close temporal association with a documented brain insult (Beghi et al, 2010)." This seizure activity would suggest that epileptic onset began and is continued while in the presence of some other form of brain injury. Unlike seizures observed in idiopathic epilepsy, these seizures are not known to have genetic links and are solely caused by injury or brain trauma.

Origin of Cryptogenic Epilepsy. Classification of cryptogenic epilepsy occurs when the cause of epilepsy cannot be identified. For example, if a patient exhibits a normal MRI scan and does not have a genetic predisposition to epilepsy, but has recurring seizures, it would likely be classified as cryptogenic. Cryptogenic epilepsy is thought to account for the majority of cases, with 50% maintaining unknown causation, followed by approximately 30%-40% due to genetic predisposition, and the remainder being attributed to symptomatic epilepsy (University of Chicago Medicine, 2019). Bearing that in mind, focusing research on this subtype allows for results that are more applicable to the population. Overall, these forms of epilepsy are vastly different in their origin, disease mechanisms, and treatment options. However, the objective of this study is to investigate the presence of sleep disruptions in cryptogenic instances.

Epilepsy and Sleep:

Sleep disorders are prevalent in cases of epilepsy, being experienced by approximately 24% to 55% of patients (Wang et al, 2018). Sleep disruptions have long been identified in patients with epilepsy, taking various forms ranging from sleeping too much to having seizures while asleep (Cheng et al, 2013; Bazil & Sirven, 2013). Due to various sleep disruption presentations, it is very unclear as to the underlying cause. Excessive daytime sleepiness (EDS) has been clearly documented in epilepsy patients with the determining factors being seizure occurrence and comorbidities (Manni & Tartara, 2000). In addition, some researchers hypothesize that sleep deprivation increases the risk of seizure activity to occur, due to a minimized REM stage, thus elevating seizure frequency (Bazil & Sirven, 2013). This research proposes that the relationship of epilepsy and sleep produces a positive feedback loop, in which a decrease in sleep yields an increase in seizures (Bazil & Sirven, 2013). Following the heightened seizure activity, there will be further disruptions in sleep, and so on (Bazil & Sirven, 2013).

Others believe that sleep disruptions can be primarily attributed to side effects produced by commonly prescribed anti-epileptic drugs (AEDs) (Bazil, 2003). In addition, it has been proposed that TLE might intrinsically carry a sleep disorder, with those patients naturally producing elevated levels of melatonin (Sammaritano, 2001; Bazil & Sirven, 2013). The results of this study could be used to help identify which proposed hypothesis is the most likely.

Neurotransmitter Modulation:

Previously, it has been noted that generalized idiopathic epilepsy and nREM sleep operate via the same thalamocortical networks (Wang et al, 2018). This would explain why many of the neurotransmitters used to regulate the sleep-wake cycle also contribute to seizure modulation. For example, the neurotransmitters adenosine, melatonin, prostaglandin D2, serotonin, and histamine are all known to have antiepileptic effects (Wang et al, 2018). Melatonin improves sleep quality, allowing an individual to spend more time in REM sleep, which blocks motor neurons and inhibits seizure activity (Li et al, 2017). The accumulation of adenosine can limit interictal spiking, which is a magnification of electrical activity between seizures (Wang et al, 2018). Adenosine is also a known mediator of the sleep cycle, with a build up of adenosine occurring during wakefulness and then a subsequent decrease during periods of sleep. Since adenosine promotes sleep, the longer the wakefulness period, the more sleep the individual is going to need to rebound (Sims et al, 2013). Additionally, the activation of serotonin receptors increases the conductance of potassium channels by elevating membrane hyperpolarizations, yielding anticonvulsant results (Theodore, 2003). Serotonin cells can also be used in sustaining wakefulness and muscular tone, but tend to be inactive during periods of sleep (Siegel, 2004). Similarly, histamine is also used to encourage wakefulness, and is often directly inhibited by GABA to promote sleep (Siegel, 2004). Histamine receptors have also shown to be antiepileptic, due to their ability to control the release of GABA, glutamate, dopamine, serotonin, noradrenaline, and acetylcholine (Bhowmik et al, 2012). Furthermore, since epilepsy is typically the result of either an abnormality in inhibitory GABA or an abundance of excitatory glutamate, the ability of histamine receptors to regulate these levels is imperative to seizure reduction (Bhowmik et al, 2012). Even though these neurotransmitters are able to help hinder seizure onset, others are responsible for initiating seizure onset.

In contrast, the neurotransmitter orexin promotes wakefulness and elevates seizure activity (Wang et al, 2018). Increased levels of orexin are observed in both patients and animal models of epilepsy, providing a possible explanation for seizure onset (Wang et al, 2018). In addition, orexin receptors in the lateral hypothalamus typically fire and activate “wake centers” in the brain to sustain wakefulness throughout the day. However, during periods of intense sleep pressures or deprivation, orexin firing is inhibited by the lateral hypothalamus via the ventrolateral preoptic area, an area known for elevated levels of GABA and adenosine (Hoyer & Jacobson, 2013). Currently, there have also been studies examining the relationship between KA-induced seizure activity and weight gain, which could also be a symptom of enhanced orexin levels (Li et al, 2017). Overall, these neurotransmitters are believed to modulate seizure activity and play a role in the sleep-wake cycle.

Temporal Lobe Epilepsy (TLE):

Seizure activity is often classified into two major groups, either exhibiting generalized or focal behavior (CDC, 2018). Generalized seizures are known for impacting both neural hemispheres, whereas focal seizures are localized to one or more specific areas (CDC, 2018). Currently, TLE is recognized as the most common form of epilepsy in humans, making it a critical focus area for epilepsy researchers (Kandratavicius et al, 2014). Even though seizure origin observed in TLE is localized to the temporal lobe, the implications of seizure activity are far-reaching (Zhao et al, 2014).When people think of epilepsy, they typically only think of the actual seizure activity, whereas in reality, TLE can cause both short and long term effects on the entire body. Studies have described the extensive effects that seizures can have on one's life including alterations in cognitive functioning, language abilities, motor control, sleep cycle disruptions, social awareness, and many others (Zhao et al, 2014).

Additionally, even though TLE is very prominent in humans, research cannot be fully obtained solely from human clinical studies, in as much as they cannot provide as much information about epileptogenesis and seizure generation (Kandratavicius et al, 2014). Moreover, in order to expand comprehension past the limitations posed by human clinical studies, animal models are frequently used. Currently, modeling TLE is most commonly achieved through using chemo convulsants such as pilocarpine and kainic acid (KA) (Kandratavicius et al, 2014).

Kainic Acid (KA) Epilepsy Model:

In the field of neuroscience, one of the most commonly used models of seizure activity in rodents is the KA model, which is composed of the following sequential steps: a craniotomy, KA injection, and implantation of EEG devices (Bielefeld et al, 2017). For this specific model, KA, a glutamate receptor agonist, is injected into the brain to induce seizure activity (Bielefeld et al, 2017). Upon injection, there is an increase in neuronal depolarizations, often resulting in recurring seizure activity (Kandratavicius et al, 2014). This model exhibits the same electroencephalographic patterns and neuropathology as epilepsy observed in humans (Lévesque, & Avoli, 2013). Kainate glutamate receptors, for which KA is an agonist, are located in various areas throughout the rodent brain but in order to model TLE seizures for our experiment, treatment subjects have received intrahippocampal administration of KA (Lévesque, & Avoli, 2013).

This specific mouse model for epilepsy has been incorporated into many different research studies analyzing the implications of seizure activity. One recent study used the KA model to demonstrate how the female estrous cycle fluctuates upon seizure activity, creating a feedback loop for seizure onset (Li et al, 2016). This study further acknowledges that seizure activity causes an irregular female menstrual cycle, in which lack of cyclicity has been accredited to decreased REM sleep. (Li et al, 2016; Hachul et al, 2010). Since REM sleep shows anti-epileptic properties, this decrease in REM sleep could be responsible for an increase in seizure frequency. This is reaffirmed by a study that noticed a relationship between the type of seizure an individual is experiencing and when it is occurring during the sleep/wake cycle during their investigation of electrical activity following unilateral hippocampal KA injections (Bragin et al, 1999). This data supports the idea that seizure generation can be modulated by extra-hippocampal structures, however, this study did not further validate these claims or offer additional explanations (Bragin et al, 1999). In multiple research studies, a potential correlation between sleep cycles and seizure activity has been noted, but supporting evidence remains severely limited, making it an unprecedented area of experimentation.

CAS Epilepsy Model:

Currently, the standard TLE seizure models being used are the KA and pilocarpine models. However, the new CAS model may be even better for research due to its tunability and reduced risk. The CAS model uses enzymatic caspases expressed via an AAV viral vector to trigger apoptosis of the cells in which it is expressed. For this research, the injections target GABAergic neurons within the hippocampus as a way to induce spontaneous seizure generation, via elimination of inhibitory GABA release. The current models exhibit correlation between the number of GABAergic neurons present and the frequency of seizure activity (Buckmaster et al, 2017). It has been identified that there are low levels or a total loss of GABAergic neurons in humans and animals that experience recurring seizures (Knopp et al, 2008) This model attempts to ablate or "turn off" GABAergic neurons within an animal model in order to provoke recurring seizure activity.

This CAS model has been shown to result in less damage to the hippocampus when used at low doses (less than 2 ul), decreasing the mortality rate and making it a more sustainable model. In one study, AAV was injected into the ventral subiculum along with diphtheria toxin (DT) to generate seizure activity, which subsided after one week in 5/6 of the mice sampled (Spampanato & Dudek, 2017). Another study used a similar methodology, however, tetanus toxin light chain (TT) was expressed rather than DT via AAV. Researchers found that initial rates of spontaneous seizures declined by 30% over the course of 5 weeks and determined that there were not any signs of hippocampal sclerosis (Drexel et al, 2017). The results of these studies are indicative of minimal damage to the hippocampus and the presence of tunability due to seizures subsiding a few weeks post injection. This tunability enables researchers to adjust seizure frequency and focal area during the experiment, allowing the same mouse to be used to create multiple models.

Research Significance:

The overall goal of this research is to determine how GABAergic neuron ablation impacts seizures activity and sleep cycle disruptions in male mice. This research only includes male mice because males have a higher likelihood of being diagnosed with epilepsy in comparison to females (McHugh & Delanty, 2008). Epilepsy has been established as a very common neurological condition, with an estimated 1 in 26 people in the U.S. being diagnosed during their lifetime (England et al, 2012). With the number of cases increasing exponentially every year, it is imperative that researchers and medical personnel have a more complete understanding of this disorder. This includes everything from the origin, disease mechanism, and effective treatments for patients. Even though epilepsy has been around for centuries, there is still a vast amount of information that is not well understood.

One of these mysteries is the connection between seizure activity and sleep. Even though sleep disturbances and seizure activity have repeatedly been noted, an explanation has yet to arise. The goals of my research are to provide clarity to this research question and expand the pool of information about epilepsy. This research studies TLE specifically, which is one of the most common forms, making obtained data highly applicable to the population.

Acknowledging the far-reaching symptoms exhibited by TLE, my research team and I wanted to analyze if alterations in sleep cycles precede seizure onset. Researchers have observed a connection between sleep disturbances and seizure onset, but it has yet to be determined whether this is merely coincidental and if it is applicable to all seizure variations (Gibbon et al, 2019). This further simplified our research question to analyzing how GABAergic neuron ablation can induce seizures in mouse models and impact the subject's sleep cycle. Additionally, this study can provide insight on the best ways to address symptoms of sleep abnormalities in patients with epilepsy. Furthermore, we hope to uncover a statistically significant relationship between seizure onset and sleep cycle disruption. In addition, we hope to identify the underlying mechanism responsible for this relationship, in hopes of making it easier to treat.

Methodology

Animals

The mice used in this experiment were Vgat-Cre mice with a genetic background of C57BL/6J. In order to be included in the study, the mice had to be between 10-15 weeks at the start of injection. The animals were fed Purina Rodent Chow throughout the duration of the experiment, once successfully weaned from its mother. The mice were maintained in a 12:12 light/dark cycle, with the lights on starting at 7am (ET) and lights off starting at 7pm (ET). Constant care was provided and the mice were attended to based on IACUC standards.

Surgeries

In all of the models prepared, 3% isoflurane was used to anesthetize the mice initially and was maintained with 1% isoflurane for the duration of the surgery. Once anesthetized, the mice were connected to a stereotaxic apparatus to carry out both the injection and EEG headcap implantation. Following the surgeries, they were given the painkiller Meloxicam at a ratio 5 mg Meloxicam to 1 kg of individual mouse weight.

CAS Model

The CAS model was generated by unilaterally injecting Vgat-Cre mice on a C57BL/6J background with AAV2-Flex-taCaspa3-2A-TEVp. The injections ranged from 0.5ul to 1.5ul and were made into the dorsal (AP: -2.53mm; ML:+2mm; DV:-1.8mm) and ventral (AP: -3.7mm; ML:+2.76mm; DV:-3mm) hippocampus. The coordinates used were from bregma and the dural surface. The injections were administered at a flow rate of 23 nl/sec and were controlled using a nanoject, with 10 pushes of the nanoject administered (10 x 50.4 nl for each injection). Injections were given while the mouse was anesthetized and preceded headcap implantation to reduce the number of surgeries being performed, limiting the time spent under anesthesia.

Kainic Acid (KA) Model

The KA model was generated by injecting Vgat-Cre mice with 0.1 ul of 20 mM kainic acid into the left dorsal hippocampus at the coordinates AP: -2mm, ML:+1.25mm, DV:-1.6mm. The coordinates used are from bregma and the dural surface. This was done while the mouse was anesthetized and preceded headcap implantation to reduce the number of surgeries being performed and limit the time spent under anesthesia.

Head Cap Preparation and Installation

The head caps were prepared and implanted using the same methodology outlined in the paper *Reconfigurable 3D-Printed Headplates* (Zhu et al, 2020). Following the 3D printing and electrical configuration of these head caps, they were surgically implanted into their respective regions. The two hippocampal tethered depth electrodes were placed at AP: -2.53mm, ML:+/-2mm, DV:-1.8mm, a frontal electrode at AP: +1mm, ML:+1.3mm, and parietal EEG electrodes at AP: -1.5mm, ML:-2.8mm. In addition, a reference screw was placed at AP: -6mm, ML:0mm, a ground screw at AP: 1mm, ML:-1.3mm, and two EMG electrodes were connected to muscles in the neck.

EEG Recording

After the mice underwent injection and surgery, they were recorded continuously for 40 days unless they died before the 40 day period elapsed. Once the post-surgery recording session ended, the mice were then documented once a week thereafter. The data acquired was from the headcap coupled with video footage.

Data Compilation and Evaluation

Once the headcap recordings were completed, the data was uploaded to the program Spike 2 version 9.10, where specific scripts of code were used to interpret the data. This program and the scripts allowed for easy identification of seizure activity, which was then cross-referenced to video recordings of the mice. Seizures were determined using EEG recordings, in which they are identified as periods of uncontrollable electrical signals, exhibited by increased frequency and high amplitude. The mice were placed in a dark room with remote controlled lights allowing for manipulation of the day/night cycles. The shifts in the light and dark conditions were manually marked using the Spike 2 application. A secondary Spike 2 script titled, “Automatic Sleep Staging for Rats” (CED, Cambride, England) was used to determine the mouse’s sleep state throughout the duration of the recording, which was then verified using the EEG recordings.

Sleep states were evaluated using a series of 20 second long epochs to determine the average time spent in each sleep state. The number of epochs in the recording were then compiled, and the percentage of time in each sleep state was generated. The script used also denotes periods of “doubt”, which were excluded from the data set and the additional values were normalized to 100%. In addition, during periods of seizure activity, the number of epochs occurring during the seizure were counted and then also normalized to 100%. Seizure frequency and sleep state duration was then analyzed over the course of 40 days. The data analyzed included seizure frequency pre/post injection, amount of time in each sleep state, and seizure duration.

Statistical Analysis

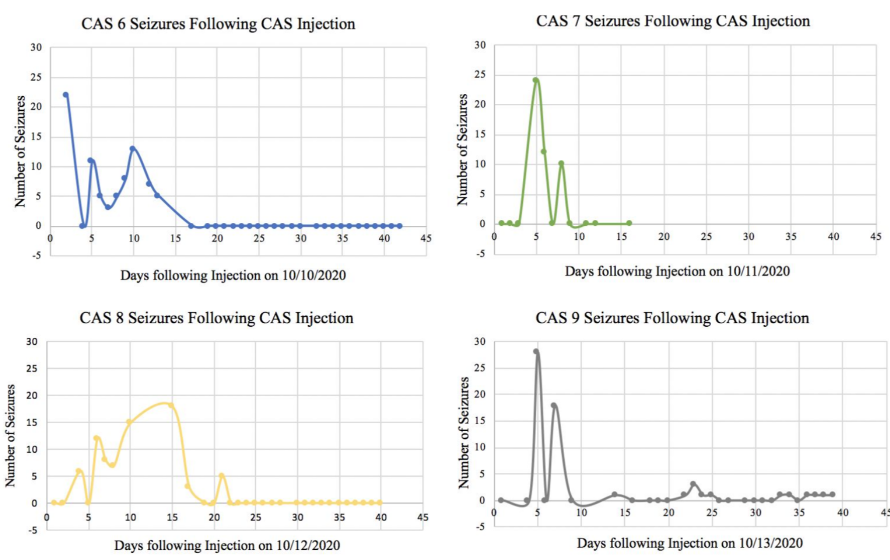
Following data collection, a series of statistical analyses were performed in order to directly compare the results of the CAS model and the control group. The program Minitab was used to generate a z-scores test which is able to identify significant data differences from a normal distribution. The alpha value used for all statistical tests was equal to 0.05, which is represented by a z-score of 1.64.

Results

Seizure Frequency following CAS injection

The results of this experiment aims to explain the feasibility of using the CAS model to effectively represent TLE. It has been hypothesized that this model might be a better model than the KA model because it might result in less damage to the hippocampus . All of these mice display an initial bout of seizure activity after receiving the CAS injection, with an average onset 4 days following ablation of GABAergic neurons. The delayed seizure onset represents the amount of time for the CAS injection to reach

Figure One: Seizure Prevalence Following CASpase Injection

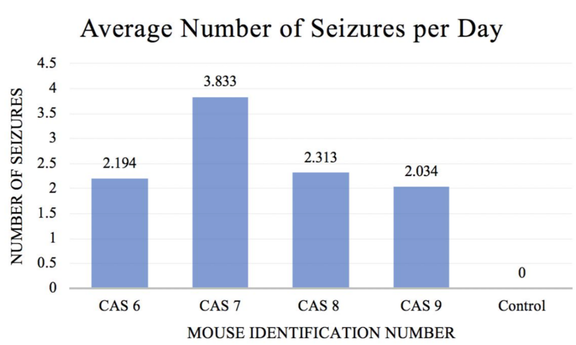


Spontaneous seizure generation has an average start at 4 days post injection and exhibits a large initial increase. This seizure period has mean duration of 12.75 days, which is then followed by a dramatic decrease back to baseline values (+/- 1 seizure). Seizures were identified using Spike Recorder Software and then cross referenced to video footage. The seizures were counted for approximately 40 days post injection, except for CAS 7, which died on 10/27/2020.

the targeted neurons and reduce GABA release to generate the seizures. Following a short period of time (mean=12.75 days), all of the mice exhibited a dramatic decrease in their number of seizures. It is thought that this drop in seizure number is indicative of the CASpase injection not causing permanent damage, but rather an acute seizure model(Figure 1).

Average Number of Seizures per day

Figure Two: Normalization of Seizure Activity per Day



The normalization of seizure activity as a function of days recorded following CASpase injection. The number of seizures per mouse was totaled and averaged across the number of days it was recorded. EEG recordings were conducted for approximately 40 days post injection, but varied slightly depending on injection date and instances of premature death. The recording durations for CAS 6 - CAS 9 were 42, 16, 40, 39 days, respectively. The average across all mouse models used is equal to 2.954 seizures per day with an average recording length of 34.25 days.

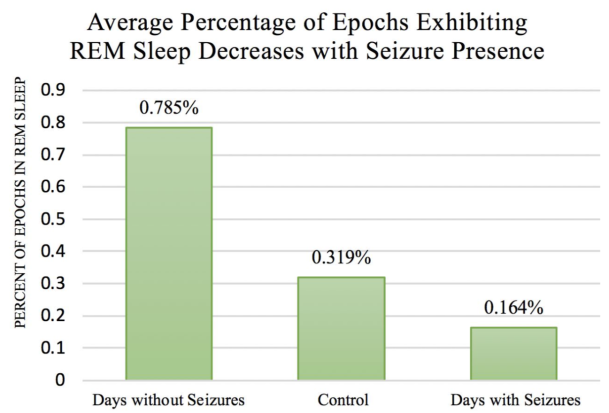
The control mice for this experiment did not exhibit any seizure activity throughout the course of this study. As compared to controls, all the CAS expressing vector injected mice developed seizures ($z = 5.19$, $p < 0.0001$) indicating the difference in the number of seizures between groups was statistically significant. In addition, the average number of seizures are used to normalize the data and account for the differences in recording duration across trials. During this experiment, CAS 7 died, likely resulting from having a significantly larger number of seizures compared to the other models (95% confidence interval=1.776, 3.4110).

Average Percentage of Epochs in REM Sleep

The average percentage of epochs in REM sleep (mean= 0.785%) for the trials occurring without seizure activity is 1.46%, 0.88%, 0.77%, 0.0311% for CAS 6 - CAS 9 (Figure 3). Days with seizures had average recording values of 0.15%, 0.31%, 0.17%, and 0.0265% for CAS 6 -

CAS 9 (mean= 0.785%). For the control data, the average REM percentage was 0.319% with a standard deviation of 0.01575. A z-score analysis was performed for seizure (z=-19.67, p <0.0001) and non-seizure (z=59.21, p <0.0001) days. Both categories have a statistically significant difference in the percentage of REM sleep compared to the control data, making it unlikely that differences are due to error. This data shows a REM sleep increase of 0.466% on days without seizures and a 0.155% decrease on days with seizures, following GABAergic neuron ablation.

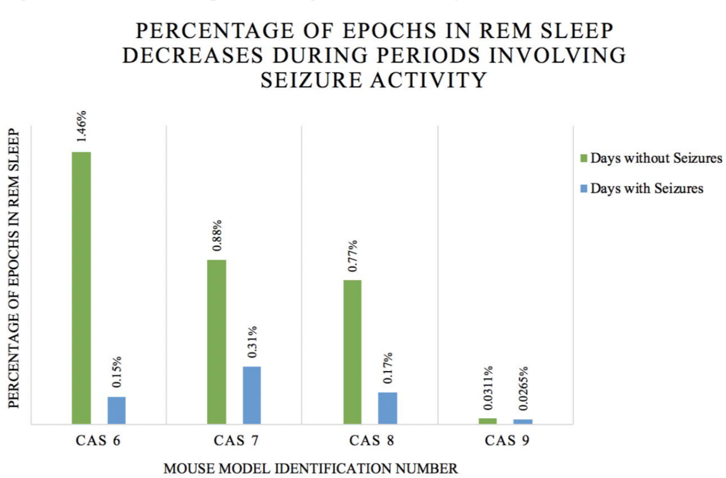
Figure Three: Sleep State Distribution and Seizure Occurrence



Average percentage of epochs exhibiting REM Sleep decreases during periods of seizure activity. The control group was injected with saline and did not exhibit any seizures. The REM sleep percentages were generated by averaging the total percent of epochs in REM sleep for all of the days that a seizure did not occur. The same was done for the days where seizures were present. A z-score analysis was performed and generated z-scores of 59.21 and -19.67 respectively.

Average Percentage of Epochs in REM Sleep by Individual Mouse

Figure Four: REM Sleep Percentage Breakdown by Individual Mouse Model



Percentage of epochs in REM sleep decreases on days when seizure activity is present. 24 hour EEG recordings were analyzed for each of the models and the percentage of 20 second long epochs in REM sleep was averaged. This was repeated for days with and without seizures. Significance was then determined using a z-score analysis with a known standard deviation of 0.1575, a control average of 0.319, and an alpha value of 0.05.

The average REM sleep percentages coupled with seizure presence are shown for the individual mouse models. The four saline controls used for this experiment yielded an average REM sleep percentage of 0.319% and standard deviation of 0.01575. This control group served as the baseline value for the z-score analysis performed. For CAS 9, the difference between the days with and without seizures is very small, whereas in the other trials, there is a very prominent difference. The REM value for CAS 9 on days without seizures is not statistically significant, however, this is not true of the remaining data (p-value<0.05). If no seizures were present during the 24 hour period, the REM sleep percentage was significantly larger than both the control and seizure groups. With more research, increased percentages of REM sleep during periods of seizure activity could potentially be used to predict future seizure occurrence.

Discussion

The results of this study indicate that there is a correlation between sleep cycle disturbances and seizure activity generated via GABAergic neuron ablation. One finding of this research is that spontaneous seizures are generated at a statistically significant rate following CASpase injection. Even though the CAS model is still new, seizure generation via GABAergic neuron ablation has been used previously in the KA model (Knopp et al, 2008). These results align with the current evidence supporting spontaneous seizure generation following GABAergic neuron ablation (Lévesque & Avoli, 2013). This is indicative that this innovative CAS model is an effective model for TLE, however, its durability long term cannot be determined due to the length of this research study only being 60 days. Future longitudinal studies could be used to establish durability and reaffirm that it is less harmful than its counterparts through histological methods.

Following GABAergic neuron ablation, the percentage of REM sleep increases on days without seizures and decreases on days with seizures. In addition, on days when REM sleep duration is elevated, seizure prevalence decreases. A possible explanation for this phenomenon could be that REM sleep acts as “protection” against seizure frequency (Bazil, 2019). Another possibility would be that seizures prevent REM sleep from occurring, yielding lower REM sleep percentages. In addition, on non-seizure days, the average percentage of REM sleep epochs was higher than the saline control group. This data supports the hypothesis that TLE intrinsically includes a sleep disorder, as suggested by prior studies (Sammaritano, 2001; Bazil & Sirven, 2013). The methodology behind why this occurs is still unknown, but it is hypothesized that increased melatonin secretion during REM sleep can act as an inhibitory neurotransmitter that reduces overactive electrical signalling.

For this experiment, the possible sources of error could be due to instrumental and human errors. One possible source of human error could be due to the manual identification of seizure activity. There are general guidelines as to how seizure activity is determined, however, various researchers may identify the occurrence and duration of the seizure differently. Another potential source of error could be the result of instrumentation. All of the head caps were handcrafted with a very intricate circuit. If one of the wires was slightly off or not signalling to the main computer correctly, an inaccurate signal could result.

This understanding can aid in larger applications such as drug design and effective epilepsy treatments. In the future, the data from this research could be expanded to pinpoint REM sleep percentages immediately prior to and following seizure activity. This could provide further insight into EEG signalling changes that occur shortly before seizure onset, which could aid in the development of seizure prediction technologies. Right now, since numerous ion channels are thought to have a role in seizure generation and epileptic conditions, it is exceedingly complicated to design effective and uniform treatments. By gaining more insight into these mechanisms, it will be easier to identify the far reaching implication of epilepsy and equip physicians with more possible treatment options and seizure prevention techniques.

Conclusion

In conclusion, despite the fact that epilepsy has been acknowledged for thousands of years, it is still not understood well. The types of seizures and epilepsy classifications vary immensely, making it extremely difficult for researchers to pinpoint specific causes of this neurological disorder. Moreover, due to the deviation in epilepsy presentation and symptoms, there is an abundance of research questions that remain to be answered. In this case, we addressed to what extent KA/AAV-induced seizures are associated with sleep cycle disruption in animal models. This understanding can aid in larger applications such as drug design and effective epilepsy treatments. Presently, it is estimated that AEDs are not effective in about 20% of those with epilepsy, making it much more difficult to manage seizure activity (Epilepsy Foundation, 2017). Additionally, researchers are still attempting to improve drug design and to gain a better insight into the mechanism behind epilepsy. As of right now, while great strides have been made in gaining an understanding of this disorder, there is still plenty to be discovered. In the future, hopefully, these scientific inquiries and ideas are brought into fruition, yielding an even more extensive understanding of epilepsy.

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