



**SEIZURE ONSET AND OFFSET DYNAMICS IN A TEMPORAL LOBE EPILEPSY
MOUSE MODEL OVER 90 DAYS**

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Abstract

Epilepsy is a disease characterized by repetitive seizures that affects 1 in 26 people, and a third of the patients are drug resistant. Activity in the brain is altered during seizures with an increase in synchrony and reduced inhibition. In order to improve the development of anti-epileptic drugs, various mouse models of epilepsy are necessary to study the disease. In this study, we specifically study the dynamics of recurrent seizures over the course of epileptogenesis in the intra-amygdala kainic acid (IAK) model of temporal lobe epilepsy. Previously recorded electroencephalograms (EEGs) of IAK mice from 90 days were observed and categorized by the shape of their seizure onset and offset, referred to as dynamotypes, based on the patterns or lack of patterns in amplitude and frequency. In both seizure onset and offset, the seizures lacked a pattern in amplitude and frequency (SubH and FLC dynamotypes) during the early stages of epileptogenesis (first 30 days). In the later stages, i.e. the last 30 days, the seizures developed patterns of amplitude or frequency in both the onset and offset dynamotypes. We hypothesize that the gradual development of patterns in onset and offset indicate more stereotyped seizure dynamics later in the course of epileptogenesis, potentially indicating a greater difficulty to treat established seizure networks with anti-epileptic drugs.

Introduction

The Wilcox lab studies the mechanisms involved in the occurrence and treatment of epilepsy and seizures. This project will be focused on classifying seizures based on the characteristics of their onset and offset characteristics, duration, and dominant frequency using electroencephalography (EEG) data recorded from the hippocampus in a IAK model of temporal lobe epilepsy. Categorizing seizures will allow further observation on seizure properties and if they change over, thus, indicating the evolution of epilepsy over time from its initial onset. Understanding the dynamic nature of epilepsy may guide the testing of anti-epileptic drugs and provide additional metrics to quantify drug efficacy.

Epilepsy is a disease that affects the neurons in the brain, disrupting its activity and causing seizures. According to the Center of Disease Control and Prevention (CDC), this disease affects 3.4 million people, both children and adults, in the United States. There are many different types of epilepsy, depending on where the seizure stems from as well as how the seizure manifests. The probability of the mice experiencing seizures after the initial status epilepticus event increases as the brain becomes accustomed to having seizures (Fisher et al., 2005). The intra-amygdala kainic acid (IAK) mouse model is an induced model of temporal lobe epilepsy where mice are injected with kainic acid in the basolateral amygdala to induce a seizure. The repeated seizures in response to kainic acid are called status epilepticus, and may lead to the development of epilepsy, which is characterized by spontaneous recurrent seizures. In a published study from the Wilcox Lab (West







et al., 2020), 24/7 video and EEG was collected for 90 days in 54 mice after kainic acid injection to quantify the onset of epilepsy and regularity of spontaneous seizures. Seizures were detected by researchers trained in EEG review and confirmed by the behavioral manifestation of an EEG seizure. The study quantified seizure severity according to the Racine scale (Racine, 1972) and looked specifically at whether or not there was a seizure over the 90-day period. However, this study did not quantify the specific temporal or frequency characteristics of each seizure.

Methods

A subset of 18 IAK mice from the West et al., 2021, study was used in this study. All mice were observed for 90 days post-injection, and 24/7 video and EEG were recorded. The files were analyzed using custom software on MATLAB and a dynamotype rating was assigned to each onset and offset period manually in a blinded fashion. Each seizure had their onset and offset dynamotypes rated based on the patterns or lack of patterns in frequency and amplitude of the first 5 seconds of the seizure and the last 5 seconds of the seizure according to Figure 1.

The seizure characteristics classified in this study are seizure duration, the dominant frequency at the seizure onset and offset, and the onset and offset dynamotypes, which classify the onset and offset patterns according to bifurcation theory.

According to the Saggio et al., 2020, there are 16 dynamotypes possible in a simplified bifurcation model with two variables. Classification of seizure onset and offset into different dynamotypes depends on the patterns in spiking frequency and amplitude changes. There are 4 onset types (Saddle-Node, Saddle-Node Invariant Circle, Supercritical Hopf, and Subcritical Hopf) and 4 offset types (Saddle-Node Invariant Circle, Saddle Homoclinic, Supercritical Hopf, and Fold Limit Cycle), which all have different patterns of amplitude and frequency that uniquely describes each dynamotype. For the purposes of our study, SH and SN, both dynamotypes that have a DC offset (the increase in the baseline frequency at seizure onset), will not be used since the recording system included a DC filter. In Saggio et al., 2020, study, they did not filter out the DC offset. The Saggio study also found that all these classifications of seizure dynamotypes are represented in human seizures, and the dominant dynamotype can change as time goes on within patients. In the proposed project, the EEG seizure recordings from the West et al. paper will be analyzed and categorized based off of the criteria described in the Saggio et al. paper in addition to seizure duration and the dominant frequency at the seizure onset and offset.

Bifurcation	Time-series	Amplitude	Frequency	Baseline Jump	Rest-Seizure
Saddle-Node (SN)		non-zero	non-zero	can be present	→
Saddle-Node Invariant Circle (SNIC)		non-zero	zero ($\sqrt{\lambda}$)	no	↔
Saddle-Homoclinic (SH)		non-zero	zero ($\ln \lambda$)	can be present	←
Supercritical Hopf (SupH)		zero ($\sqrt{\lambda}$)	non-zero	no	↔
Subcritical Hopf (SubH)		non-zero	non-zero	no	→
Fold Limit Cycle (FLC)		non-zero	non-zero	no	←

Adapted from Saggio et al., 2020, *eLife*

Figure 1. There are 3 different onsets, SupH characterized by increasing amplitude, SNIC characterized by increasing frequency, and SubH characterized by abnormal patterns in both amplitude and frequency. There are 3 different offsets, SubH characterized by decreasing amplitude, SNIC characterized by decreasing frequency, and FLC characterized by abnormal patterns in both amplitude and frequency.

Results

Throughout the 90-day course of the study, the mice injected were observed and their seizures were recorded. We visualized the seizures over the course of the study for each mouse in a heat map, as shown in figure 2. The mice had a median of 22 seizures, a 25th percentile of 7, 75% of

28, minimum of 1, and maximum of 294 seizures. As the study progressed, the mice started to have an increase in the number of spontaneous seizures they experienced. During the first 30 days the mice had 86 seizures, in the middle 30 days they had 242, and in the as 30 days they experienced 365 seizures.

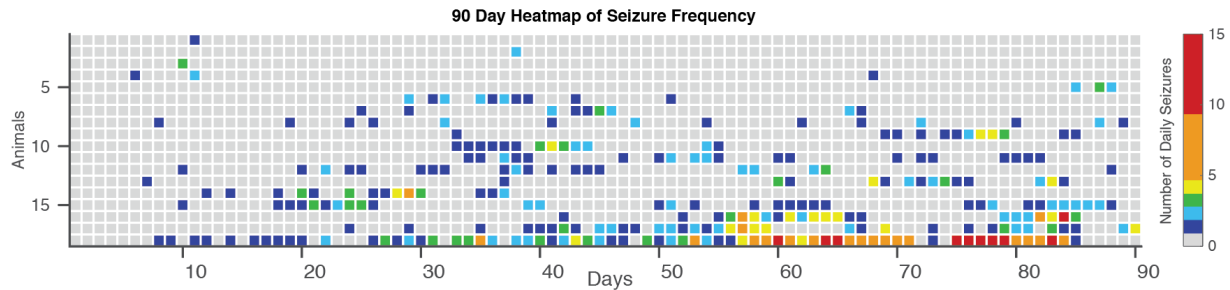


Figure 2. The heatmap depicts the total number of spontaneous seizures of each mouse over 90 days post-kainic acid injection. The mice were ranked by the lowest number of total seizures to the highest, from top to bottom. Seizure totals: Median = 22; 25% = 7; 75% = 28; Minimum = 1; Maximum = 294.

The 18 mice were then sorted into two different groups, the early latent period and the late latent period. The group they were put into depended on their latent period, or when they had their first seizure. The early latent group consisted of the mice that had their first seizure within the first

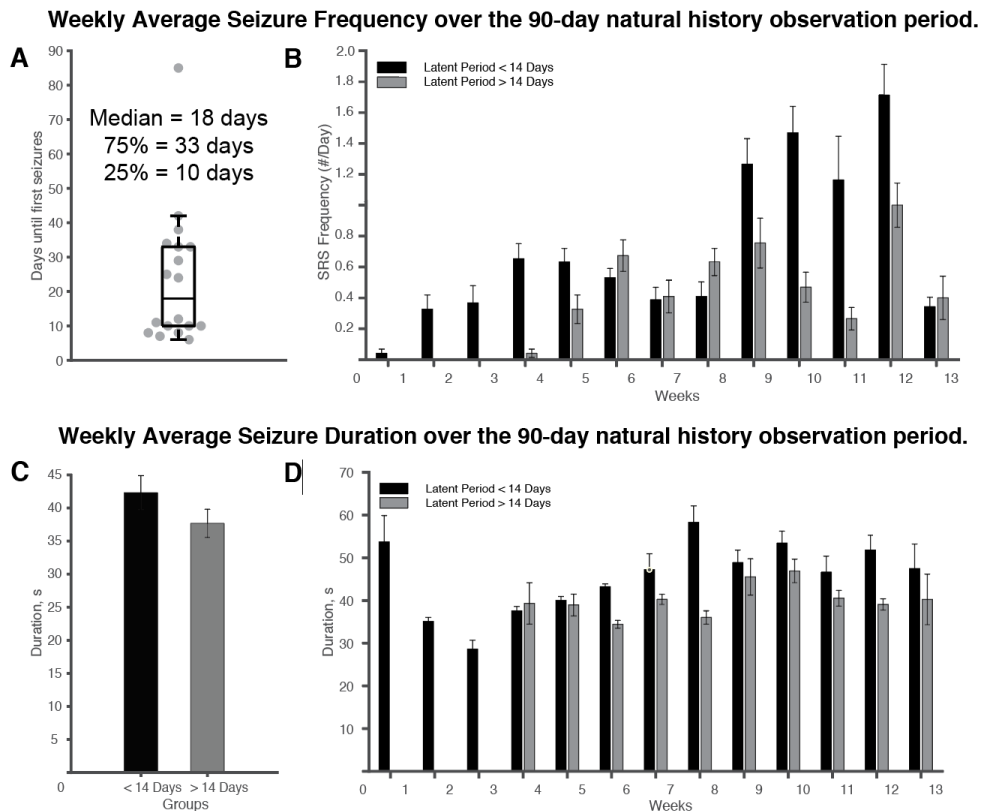


Figure 3. **A.** Mice with kainic acid injections experienced spontaneous seizures at a median of 18 days after status epilepticus. **B.** Over the 90-day period, daily seizure frequency was not significantly different across early and late latency groups (t-test, $p = 0.3822$). **C.** Mice in the early latency group had average seizure durations of 42.3 seconds. Mice in the late latency groups had a mean seizure duration of 37.7 seconds. These seizure durations were not significant between groups (t-test, $p = 0.113$). **D.** Seizure durations are stable over time across both groups.

14 days of the study, while the late latent group consisted of mice that had their first seizure after the 14-day mark. The median latent period was 18 days, the 75th percentile was 33 days, and the 25th percentile was 10 days (Figure 3A). These two groups were then compared to determine if there were any differences regarding average seizure frequency and average seizure durations (Figure 3B-D). There were no significant differences found among the two groups.

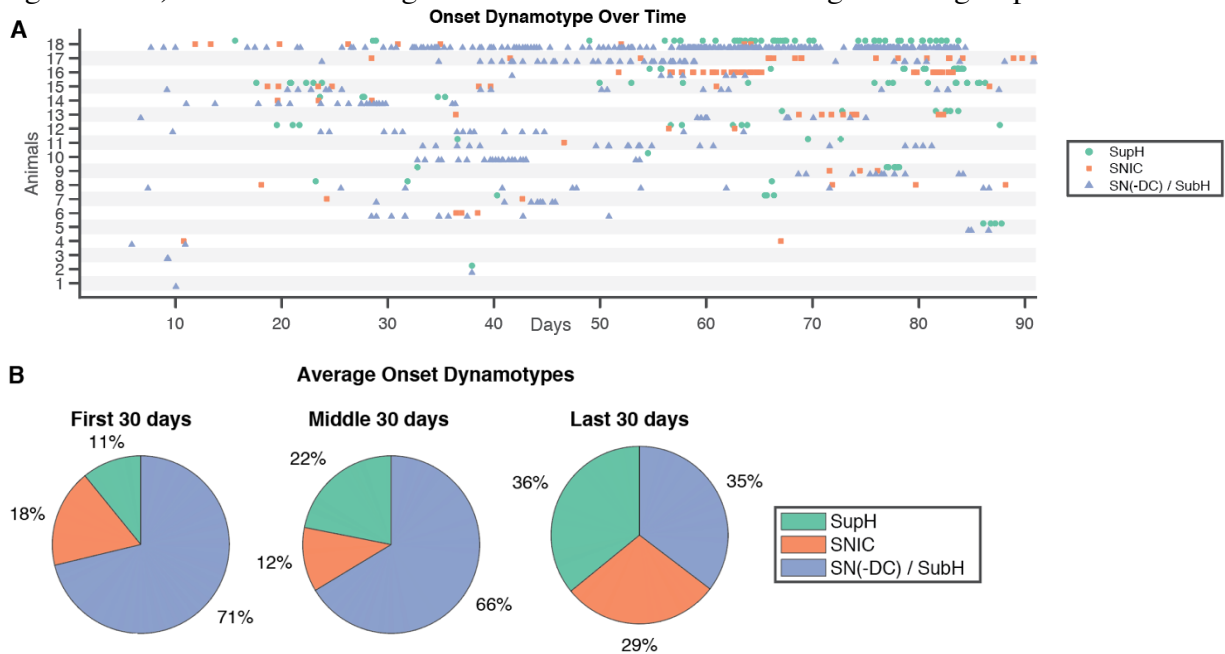


Figure 4. **A.** Early in epileptogenesis, the onset dynamotype of seizures were dominantly SubH, characterized by abnormal patterns in frequency and amplitude. As time progressed, there were more SNIC (increasing frequency) and SupH (increasing amplitude) onset dynamotypes. **B.** The average onset dynamotypes in the first 30 days are dominated by SN(-DC) or SubH (71%). In the last 30 days, all onset types are represented approximately equally. SN(-DC) or SubH was significantly reduced from the first 30 days to the last 30 days (ANOVA, $p = 0.0183$).

All of the seizure onsets over the course of the study were recorded and depicted in figure 4A. At the very beginning of epileptogenesis, the majority of the seizure onsets lacked any patterns in both frequency and amplitude (SN(-DC)/ SubH dynamotype). As time went on, the seizure onsets started to have a pattern of increasing amplitude (SupH) and a pattern of increasing frequency (SNIC). Figure 4B shows how the average onset dynamotypes changed over the 90 days. The first 30 days show that 71% of the onset was the SN(-DC)/SubH dynamotype, and by the last 30 days, this decreased to 35% which was a significant change. SupH and SNIC started at 11% and 18%, and this increased to 36% and 29% in the last 30 days.

Seizure offsets were graphed over 90 days in figure 5A. The seizures at the beginning of the study had the FLC dynamotype, which is characterized by abnormal patterns in both amplitude and frequency. The offset dynamotype started to change into Sup H (decreasing amplitude) and SH(-DC)/ SNIC (decreasing frequency) as the study continued. According to figure 4B, 79% of the offsets were FLC in the first 30 days, this decreased to 43% in the last 30 days, which was a significant change. SupH and SH(-DC)/ SNIC started at 9% and 12% in the first 30 days, and then increased to 23% and 34% in the last 30 days.

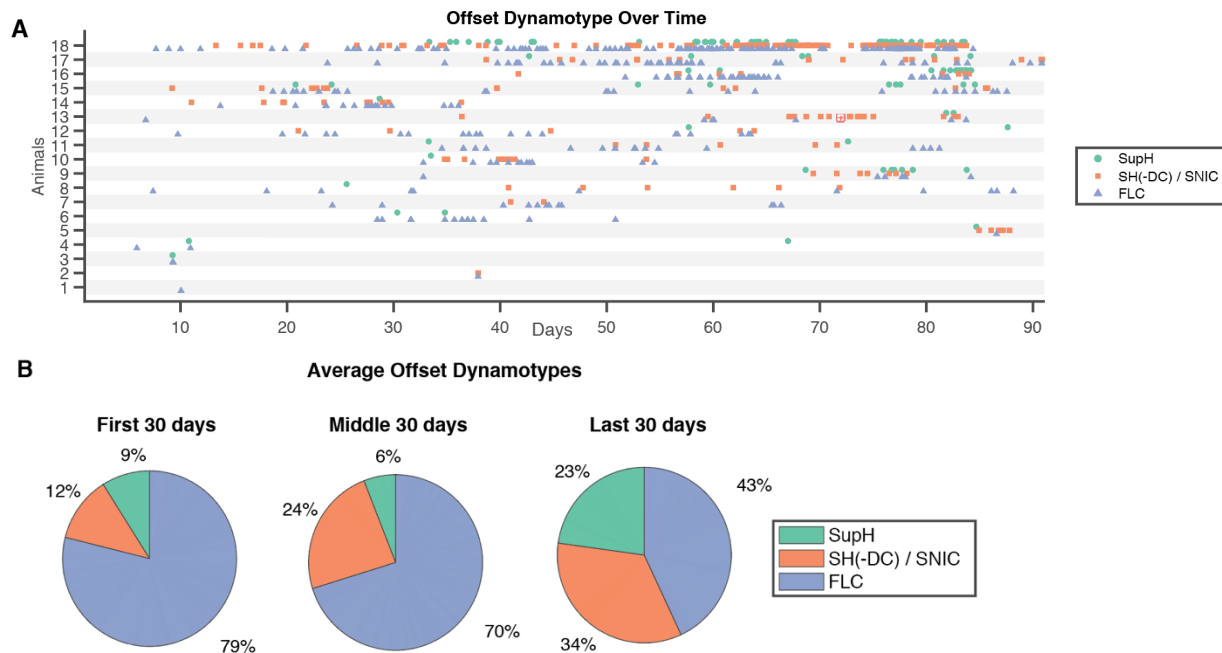


Figure 5. A. The offset dynamotypes were dominantly FLC at first, characterized by abnormal patterns in frequency and amplitude. As time progressed, SNIC (decreasing frequency) and SupH (decreasing amplitude) were more prevalent dynamotypes. B. The average offset dynamotypes inn the first 30 days are dominated by FLC (79%). In the last 30 days, the prevalence of SH(-DC) or SNIC and SupH increased. FLC was significantly reduced from the first 30 days to the last 30 days (ANOVA, $p = 0.0005$).

Discussion

In the IAK model of mesial temporal lobe epilepsy, epilepsy was induced in 18 mice following an injection of kainic acid into the basolateral amygdala. The mice were then observed for 90 days, during which 24/7 EEG and video were recorded. These seizures that were observed by the mice were categorized by dynamotype, which is based on the patterns of seizure onset and offset seen at the first 5 seconds of the seizure, and the last 5 seconds of the seizure. These patterns were tracked during the 90 days in order to observe any changes with onset and offset.

The mice had seizures that lacked any consistent pattern in amplitude and frequency at the beginning of the study. As time progressed, the seizures started to develop patterns in amplitude and frequency in both the seizure onset and offset. This finding was similar to what was seen in the Saggio paper, in which they observed every type of dynamotype within the patients. They also found that as the study progressed, the dominant dynamotype within the patients changed. We hypothesize this increase in patterning of onset and offset dynamotypes later in the course of epilepsy indicates more stereotypes seizure networks over the course of epileptogenesis. This progression of seizure dynamics and the changes in the brain over time was also seen in other studies (Crisp et al., 2020).

We will further test whether seizure frequency, duration, or severity correlate with onset and offset dynamotypes. In addition, we will test to see how dynamotypes may change in the presence of anti-epileptic drugs such as phenobarbital (which significantly reduces seizures) and phenytoin (which does not significantly reduce seizures). We would like to test to see if a specific drug is more effective when there is a specific dominant dynamotype present. Antiepileptic drugs have

the ability to change the state of the brain and the seizure dynamics that are present (Crisp et al., 2020). If drugs have the ability to change the dynamotype into a different dynamotype, we can test if one could be easier to treat than another. Different seizure onset characteristics are also associated with different pathologies (Perucca et al., 2013). Knowing this, we could further test to see if certain drugs are able to better treat epilepsy that was linked to specific pathologies.

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