Full Documentation

1 Detection algorithm for epileptiform events from acute seizure models

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1.1 Abstract

The electrographic seizure detection algorithm (herein referred to as the "algorithm") is programmed to mimic how human experts detect the onset and offset of epileptiform events from single channel local field potential (LFP) recordings. The algorithm extracts specially engineered features and uses unsupervised machine learning (a branch of artificial intelligence) to classify epileptiform events (and artifacts) detected from the acute seizure models presented in Chang et al., 2019. The ictal events detected and classified by the algorithm were >90% in agreement with human experts. Furthermore, the electrographic seizure onset times detected by the algorithm were within 1s of the annotations by human experts. Lastly, the algorithm was fast, required no training, and user-friendly for life science researchers. These results indicate that the algorithm can be used to automate the time-consuming task of annotating epileptiform events in long LFP recordings, and ultimately expedite preclinical seizure research.

1.2 Technical Summary

The algorithm works in three stages: Detection → Feature Extraction → Classification. First, the algorithm isolates the baseline activity of the LFP recording and locates all the epileptiform spikes. The algorithm operates on the fundamental basis that epileptiform events are comprised of spikes, either individual spikes (i.e., interictal spikes) or a collection of spikes (i.e., interictal events, interictal bursts, or ictal events). Epileptiform spikes that are near each other (i.e., 10 s) are grouped as the same event. A specially designed 'crawler' function then locates the exact onset and offset times for the event, according to the methods described in Chang et al., 2018. Second, the algorithm extracts two features that are specifically engineered to capture the unique temporal dynamics of epileptiform events from *in vitro* brain slices. Third, the algorithm uses unsupervised machine learning, k-means clustering (where k = 2), on these engineered features to classify the detected events, either as an ictal event or interictal event. Many fail-safe mechanisms (i.e., removal of artifacts, minimum duration, soft-coded thresholds, and hard-coded thresholds, and others) are built on top of the k-means clustering algorithm to prevent misclassification, and ensure highly accurate labelling, in a wide range of situations.

For full details on the algorithm's code and functions, please read "Full Documentation" on *https://github.com/Valiantelab/ChangValiante2018*.

1.3 Introduction

The recent development of acute seizure models that utilize optogenetic tools to investigate seizure onset mechanisms (Chang et al., 2018) has created a newfound demand for seizure detection algorithms that can identify the exact onset of ictal events from single-channel recordings. Such a seizure detection algorithm has never previously existed for two reasons:

First, seizure detection algorithms were originally developed to assist clinicians (epileptologist) with the strenuous task of scanning through days of electroencephalogram (EEG) recordings to identify electrographic seizures (ictal events). As such, most seizure detection algorithms are developed for the clinical purpose of analysing multi-channel recordings for the presence of ictal events (*https://www.kaggle.com/c/seizure-detection*; #1 requirement for evaluation). Consequently, there are few state-of-the-art seizures detection algorithms that be reliably used to analyse single-channel recordings, especially from acute seizure models. There is also no real need for seizure detection algorithms when studying acute seizure models because the related EEG recordings are relatively short (1–2 hours) in duration. Second, seizure detection algorithms are not designed to locate the exact moment of seizure onset, as that information has little utility for the clinical purpose of confirming seizures. The state-of-the-art seizure detection algorithms are explicitly designed to detect seizure onsets with latencies up to 15 s (*https://www.kaggle.com/c/seizure-detection;* #2 requirement for evaluation). In fact, the best theoretical latency for detecting seizure onset by state-of-the-art seizure detection methods is 1 s, but in practice the best latency that could be achieved was ~3 s (Truong et al., 2017).

This paper presents a seizure detection algorithm purpose-built for identifying the exact onset and offset of ictal events from acute seizure models presented in Chang et al., 2019. The algorithm was developed to detect light-triggered ictal events from VGAT-ChR2 mice, as these events had onsets that can be considered ground truth. The algorithm was then tested with spontaneous ictal events from wild-type (WT) C57/Bl6 mice. The results indicate that the algorithm had a sensitivity of 93%, a specificity of 97%, and an accuracy of 95%. Moreover, the onset and offset of spontaneous ictal events detected by the algorithm and human experts only differed by an average of 0.83 seconds and 1.60 seconds, respectively. In comparison to all available seizure detection algorithms in the literature, this algorithm is distinct in its ability to detect ictal events from single channel recordings, unsupervised, and with a latency of >1 s.

1.3.1 Overview of the Epileptiform Event Detection Algorithm

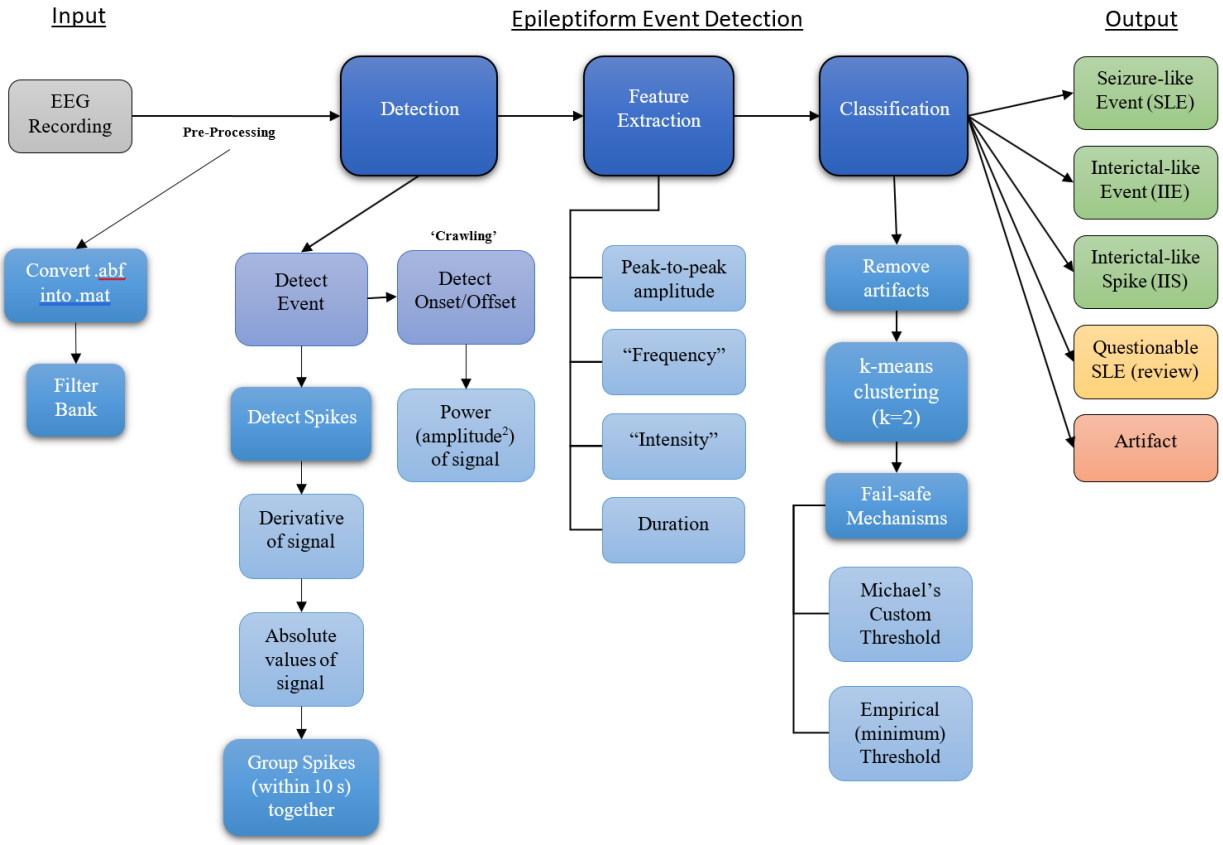
The algorithm detects epileptiform events from single-channel recordings and classifies them as an ictal event (electrographic seizure), questionable ictal event (that requires human verification), interictal event (IIEs), or artifact. The algorithm detects epileptiform events using the fundamental basis that epileptiform events are comprised of spikes. For example, ictal events and IIEs are effectively a collection of spikes, while interictal spike (IISs) are a single individual spike. Accordingly, the algorithm uses a peak finder function (*findpeaks.m)* to locate the peak of spikes in the LFP recording that cross a set threshold. The threshold is dynamically defined as (baseline's mean) + (baseline's sigma*3.9), as similarly used by other to detect epileptiform discharges (Zhang et al., 2019). A specially designed function, *crawler.m*, then locates the exact onset and offset times for epileptiform events using the same methodology as human experts.

It was discovered that there are four features ideal for classifying epileptiform events in single-channel recordings: 1) "frequency" of population spikes, 2) average power ($amplitude^2$/duration), 3) duration and 4) peak-to-peak amplitude. The algorithm classifies epileptiform events based on each of these four features, using a modified version of k-means clustering, where k = 2 (ictal events and IIEs). If a detected event is grouped into the cluster with larger values for all three of the following features: frequency, average power, and duration, it is classified as an ictal event; otherwise, it is classified as an IIE. However, if a detected event is grouped into the cluster with larger values for peak-to-peak amplitude it is classified as an artifact. The modified k-means clustering algorithm operates in two steps. First, the standard k-means clustering, where k = 2, is performed to determine the de facto threshold separating events into two clusters for each feature (i.e. frequency, average power, or duration). Second, the de facto threshold is compared to alternative thresholds (i.e., pragmatic soft-coded thresholds and empirical hard-coded thresholds) to ensure the best threshold is selected for each situation.

The algorithm is fast, unsupervised, and requires no prior training with labelled data. It can analyse one hour of recorded (single channel) data in one minute; it achieves this by limiting intense calculations to small portions of the time series (i.e. when locating the exact point of event onset/offset). The algorithm also has a modular design, so only the *crawler.m* function (which locates the exact onset/offset) needs to be modified to detect specific events from other seizure models or clinical EEG recordings.

1.4 Methods

The strategy upon which the algorithm was designed allows it to be fast and require no prior training. The algorithm is user-friendly for life science students and researchers with minimal computer programming experience. The algorithm is run as a MATLAB script, which is comprised of three stages: Detection → Feature Extraction → Classification (Appendix Figure 1). Each stage is a separate module (MATLAB function) so advanced users with programming experience can modify modules for different seizure models. The algorithm's sources code, MATLAB scripts, and detailed instructions for operation are available for download from *https://github.com/Valiantelab/ChangValiante2018* and a *video tutorial* is also available.



Appendix Figure 1: Flow Diagram of epileptiform event detection algorithm. "Frequency" is population spike rate; "intensity" is the average power (amplitude^2/duration).

1.4.1 Stage 0: Signal Pre-Processing

The raw signal (.abf files) is processed in three sequential steps. First, the signal is bandpass filtered 1–100 Hz. Second, the filtered signal is processed into absolute values and called the "absolute signal". Third, the derivative of the absolute signal is calculated to form the "derivative signal". Each signal is converted into a time series and analysed by the algorithm.

1.4.2 Stage 1: Detection

The algorithm operates on the fundamental basis that all epileptiform events are essentially a collection of epileptiform spikes. The algorithm detects spikes in the signal using the *findpeaks.m* function in MATLAB. The spikes that are within 10 s of each other are considered to be from the same epileptiform event (Lillis et al., 2012). While spikes that are >10 s apart represent the offset and onset of neighbouring events. Following this general principal, the algorithm can reliably detect epileptiform events in three stages. First, it quickly approximates the location of putative events and artifacts in the derivative time series. It achieves this by locating all the spikes that have an amplitude greater than the average value of the respective time series + the 1st quartile value*20. The algorithm assumes that all the spikes within 10 s of each other are from the same epileptiform event. Second, these putative epileptiform events and potential artifacts are then removed from the absolute time series to isolate its respective baseline signal. The baseline voltage activity is then used to calculate the thresholds for detecting epileptiform spikes and artifacts in the absolute signal. The algorithm determines the epileptiform spike threshold as the average baseline voltage activity + sigma of baseline voltage activity*hyperparameter defined by end user. The optimal hyperparameter was found to be 3.9 for non-noisy *in vitro* recordings, as similarly found by others (Zhang et al., 2019). However, larger hyperparameters (~10) were better for noisier recordings or *in vivo* recordings. Any spike greater than the average baseline voltage activity + sigma of baseline voltage activity*70 is taken to be artifacts in the recording and removed from the signal. The remaining spikes are taken to be epileptiform spikes. Thus, any spikes within 10 s of each other are grouped as a single event. Third, the algorithm detects the exact onset and offset for each epileptiform event in the absolute time series (after a lowpass filter at 25 Hz), using the rules defined by Chang et al., 2018. This is achieved by using a specially designed function, *crawler.m*, to analyse the first and last spike of each epileptiform event. The onset is determined to occur when the signal's power (feature) increases above a threshold set at 5% of the first spike's

maximal power. The offset is determined to occur after the last spike, when the signal's power decreases below a threshold set at 50% of the baseline activity's average power.

1.4.3 Stage 2: Feature Extraction

Domain knowledge of ictal events from the *in vitro* 4-AP model (Chang et al., 2018) was used to engineer new features that were effective in capturing the temporal aspects of ictal events (voltage activity). In total, the algorithm extracts four main features: "frequency", average power, duration, and peak-to-peak amplitude. The algorithm uses these four features to classify the detected events as either an ictal event, IIE, or artifact. The algorithm extracts two additional features: "tonic phase" and "intensity ratio" to confirm the classification of ictal events and IIEs, respectively.

A1.4.3.1 Engineered Features (average values)

Ictal events are distinguishable from IIEs because their population spikes (deflections in the LFP) occur at a higher frequency (>1 Hz) and a higher power (>15 mV^2/s). Thus, two features were engineered to capture those aspects of the ictal events in single channel recordings:

1) **Average frequency**, defined as total population spikes during event/duration of event (herein referred to as "frequency").
2) **Average power ("intensity")**, defined as total power of event/duration of event, where total power = $\sum amplitude^2$.

A1.4.3.2 Raw Features (total values)

Interictal spikes and artifacts also have a high frequency and high average power. For this reason, they can also be misclassified as an ictal event. Duration is used as a third feature to filter out shorter IIEs and interictal spikes that may have been misclassified as an ictal event. Peak-to-peak amplitude is used as a fourth feature to filter out high amplitude artifacts, which are significantly (i.e., 15x) larger than physiological spiking activity in the LFP recording.

3) **Duration**, length of the epileptiform event.
4) **Peak-to-peak amplitude**, the absolute difference between the maximum amplitude and the minimum amplitude of an event.

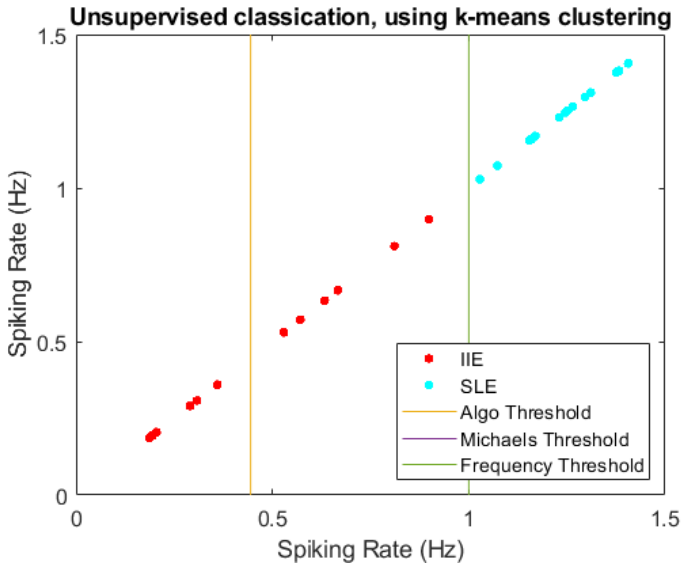
A1.4.3.3 Additional Engineered Features (per second values)

The key features that distinguish ictal events from IIEs, and IIEs from IISs are:

5) **Tonic phase**, the frequency (population spike rate) at each second through the event.
    - Ictal events must have a tonic phase, or tonic-like firing, which is high frequency (>1 Hz) population activity that is consistently maintained for at least 2 seconds.
    - Any ictal events lacking a tonic phase are reclassified as an IIE
6) **Intensity Ratio**, the proportion of time an event demonstrates high power (amplitude^2)
    - The threshold for "high power" is 0.1*the maximum power observed during event
    - The intensity ratio is the total time an event is at "high power"/total time of event.
    - In general, ictal events have a greater intensity ratio than IIEs. This feature can also be used to reclassify IIEs as questionable ictal events (QSLEs) or IISs.

1.4.4 Stage 3: Classifier

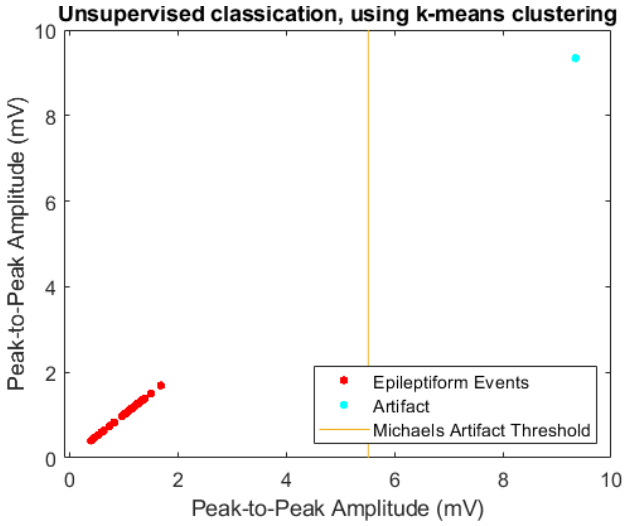
The algorithm will classify the detected events, as either ictal events or IIEs based on three features: average frequency, average power, and duration. This is achieved with a specially designed *classifier_dynamic.m* function, which performs multi-criteria decision analysis (MCDA) to label epileptiform events as ictal events or IIEs. The MCDA produces a bespoke threshold for each feature set, which can be used to divide a population into two groups. The group of events with larger values (for a respective feature set) is classified as putative ictal events ("SLE"; Appendix Figure 2). If an event is classified as a putative ictal event according to all three features, it is labelled as an ictal event; otherwise, it is labelled as an IIE. However, if any event clusters with the larger values for peak-to-peak amplitude, it is labelled as an artifact.



Appendix Figure 2: The algo-determined threshold (orange line) is the widest gap in the feature set (i.e. spiking rate) that is either at or below the gap created by k-means clustering, whichever is the lower value. However, since both the Algo Threshold and Michael's Threshold were below the minimum acceptable threshold, the hard-coded "frequency threshold" was used to classify epileptiform events as a IIE (red dots) or SLE (blue dots).

A1.4.4.1 Removing Artifacts

The algorithm will initially classify the detected events, as either epileptiform events or artifacts based on its peak-to-peak amplitude. Any events that are an outlier for the peak-to-peak amplitude feature is immediately removed from the dataset (Appendix Figure 3).



Appendix Figure 3: The peak-to-peak amplitude threshold for artifacts (orange line) is based on where the widest gap in the dataset is observed ("Michael's Artifact Threshold").

A1.4.4.2 Multi-criteria decision-making process to segment population

The algorithm segments a population of events into two groups by using a mixture of 1) unsupervised machine learning algorithms, 2) soft-coded dynamic threshold generated from population data, and 3) hard-coded thresholds based on empirical observations. The multi-criteria decision-making process for how the algorithm derives a tailored threshold to segment the population of events into two groups, based on a respective feature set, is detailed as follows:

1) Unsupervised Machine Learning
    - K-means clustering, where k = 2, is performed to determine the de facto threshold that separates epileptiform events based on a feature set.
    - The widest gap between data points for a feature set is used as the threshold if it is below than the de facto threshold determined by k-means clustering.
    - The threshold derived from unsupervised machine learning is referred to as the "Algo Threshold" (Appendix Figure 2)
2) Soft-coded thresholds
    - Pragmatically developed while training the algorithm to detect *in vitro* ictal events from Chang et al., 2018.
    - The soft-coded threshold is based on population statistics (i.e. mean–sigma*2 or sigma, whichever value is higher is used)
    - The soft-coded threshold is used if it is lower than the algo threshold derived from unsupervised machine learning; this improves the sensitivity of the algorithm.
    - Soft-coded thresholds are referred to as "Michaels Threshold"
3) Hard-coded thresholds.
    - The hard-coded thresholds are based on the floor (minimum) and ceiling (maximum) values empirically observed for each feature set (Appendix Table 1)
    - These floor and ceiling values are hard-coded into the algorithm and used to determine what the acceptable values (limits) are for each feature set.
    - If both the Algo Threshold and Michaels Threshold are lower than the floor value for a feature set, the hard-coded threshold is used, serving as a fail-safe.
    - The hard-coded thresholds are also used by default if less than six events are detected by the algorithm.
    - Hard-coded thresholds are referred to as "[feature name] threshold"

Appendix Table 1: The statistics for the ictal events' features observed in the training dataset (n = 124, N = 7 .abf files from Chang et al., 2018). "Duration" is the amount of time an ictal event exists; Frequency" is the average population spikes per second; "intensity" is the average power; "amplitude" is the max peak-to-peak amplitude.

FEATURE	FLOOR	CEILING	AVERAGE
DURATION	12.84	194.00	67.41
FREQUENCY	1.03	4.00	2.35
INTENSITY	16.21	76.28	32.43
AMPLITUDE	0.58	2.14	1.22

The algorithm's ability to compare different thresholds ensures the most suitable threshold is selected for each feature set, in a wide range of situations. An optimized threshold enables the algorithm to segment the population of events with minimal classifications errors.

A1.4.4.3 Confirming labels for ictal events and IIEs

The algorithm will use two additional engineered features: "tonic phase" and "intensity ratio" to confirm the labels for ictal events and IIEs. Any ictal events lacking a "tonic phase" are relabelled as an IIE; this improves the specificity of the algorithm. Any IIEs that demonstrate a high "intensity ratio", which is above the lowest "intensity ratio" observed for the labelled ictal events in the dataset, are reclassified as QSLEs and recommended for review by human experts.

1.5 Results

1.5.1 Training Data

The algorithm was trained ('developed') using light-triggered ictal events from seven .abf files from Chang et al., 2018. These files were labelled by human experts under the supervision of an epilepsy neurosurgeon and neurologist. Furthermore, these ictal event onsets can be considered ground truth because they were triggered on-demand (within 30 ms) by optogenetic stimulation of GABAergic interneurons (Chang et al., 2018). The algorithm that was developed reliably detected 99% (123/124) of the ictal events (from the training dataset) that were detected by human experts (Appendix Table 2). Remarkably, the algorithm detected an additional 4 ictal events that were missed by human experts. The algorithm also made 1 false positive detection (an IIE that was

misclassified as an ictal event). Collectively, the developed algorithm had a sensitivity of 99%, a specificity of 100%, and an accuracy of 100% when annotating electrographic seizures (ictal events) from the training data; calculations were performed as previously described (Zhou et al., 2013). Moreover, the ictal events' onset and offset detected by the algorithm differed from human markings on average by 0.43 seconds and 10.62 seconds, respectively. However, a side-by-side comparison of the annotations made by human experts and the algorithm revealed that the algorithm was more accurate at detecting the ictal event onset.

Appendix Table 2: Statistics on the results of the algorithm analysing light-triggered activity from *in vitro* 4-AP cortical seizure model from optogenetic VGAT-ChR2 mice. "True Positive" is the number of electrographic seizures epochs (ictal events) identified by both algorithm and human expert; "True Negative" is the number of electrographic nonseizures (interictal events) identified by both algorithm and human expert; "False Negative" is the number of ictal events missed by the algorithm, but identified by human expert; "False Positive" is the number of ictal events incorrectly identified by the algorithm; "Total SLEs" is the total number of ictal events that were identified by human experts.

FILENAME	TRUE POSITIVE	TRUE NEGATIVE	FALSE NEGATIVE	FALSE POSITIVE	TOTAL SLES
13226009	15	13	0	0	15
13227004	19	15	1	0	20
13725002	22	8	0	1	22
13725005	20	35	0	0	20
14609000	14	19	0	0	14
16125003	23	93	0	0	23
16201017	10	129	0	0	10
TOTAL	123	312	1	1	124

1.5.2 Testing Data

The algorithm was tested on spontaneous ictal events from six different .abf files from Chang et al., 2018. These observed spontaneous epileptiform events were labelled by human experts under the supervision of an epilepsy neurosurgeon and neurologist. The algorithm detected 93% of the ictal events that were detected by human experts (107/115 events from six .abf files), plus an additional 12 ictal events that were missed by human experts. Unfortunately, the algorithm made 3 false positive detections (IIEs with large amplitudes were mislabelled as ictal events). Collectively, the algorithm had a sensitivity of 93%, a specificity of 97%, and an accuracy of 95%

when annotating electrographic seizures (ictal events) from the testing data. Moreover, the onset and offset of spontaneous ictal events detected by the algorithm and human experts only differed by an average of 0.83 seconds and 1.60 seconds, respectively.

Appendix Table 3: Statistics on the results of the algorithm analysing spontaneous activity from *in vitro* 4-AP cortical seizure model from WT C57/Bl6 mice. "True Positive" is the number of electrographic seizures epochs (ictal events) identified by both algorithm and human expert; "True Negative" is the number of electrographic nonseizures (interictal events) identified by both algorithm and human expert; "False Negative" is the number of ictal events missed by the algorithm, but identified by human expert; "False Positive" is the number of ictal events incorrectly identified by the algorithm; "Total SLEs" is the total number of ictal events that were identified by human experts.

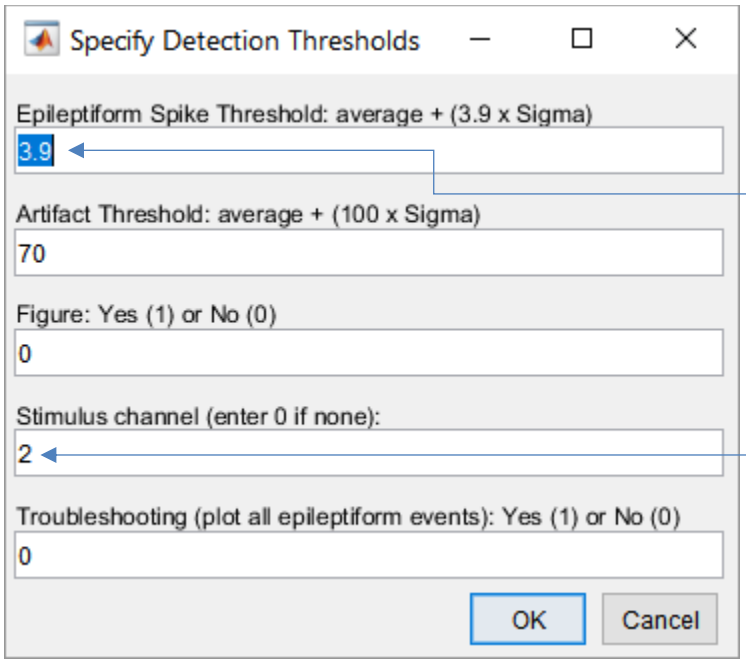
FILENAME	TRUE POSITIVE	TRUE NEGATIVE	FALSE NEGATIVE	FALSE POSITIVE	TOTAL SLES
13802004	30	39	3	0	33
13802006	20	13	0	3	20
13803004	20	4	0	0	20
13805000	8	16	1	0	9
14528001	14	12	2	0	16
14528003	15	14	2	0	17
TOTAL	107	98	8	3	115

1.6 Conclusion

The algorithm was fast, reliable, and effective at detecting ictal events (and their onsets) from the *in vitro* 4-AP seizure model. Moreover, the algorithm detected ictal event that were >90% in agreement with human encephalographers, which is considered perfect (Gaspard et al., 2014). This seizure detection algorithm combined with the acute seizure models capable of initiating ictal events on-demand (Chang et al., 2019) can form a novel ecosystem for rapid preclinical seizure research. The ecosystem can be used to effectively study seizure onset mechanisms, rapidly test new antiseizure therapies/drugs, and study critical state changes.

Appendix A Detection Algorithm Inputs & Outputs

A.1 Seizure Detection Program Input



Set the threshold to detect epileptiform spikes

The value is a multiple of the standard deviation of the recorded voltage activity.

Tip: For noisy data, use a high value, which sets the threshold higher, and effectively ignores the small spikes due to noise.

Indicate the channel of the applied stimulus

I.E. "2" if it is the second recorded channel; "3" if it is the third recorded channel; etc.).

The detection algorithm detects the peak of spikes in the LFP recording, where spikes are defined as deflection above the threshold (known as the hyperparameter). The hyperparameter is optimally defined as baseline's average amplitude + 3.9 x sigma, as previously used by other authors to confirm epileptiform discharges (Zhang *et al.* 2019). However, a higher hyperparameter may be required for noisier datasets.

A.2 Seizure Detection Program Output (Excel File)

Column:

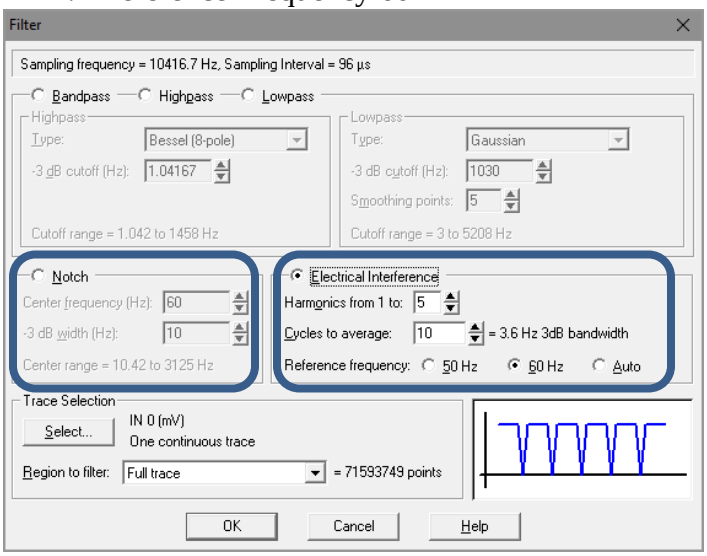
A. The onset time (seconds) for the event
B. The offset time (seconds) for the event
C. Duration (seconds) for the event
D. Spike Rate (Hz); the average number of spikes detected per second, during the event
E. Intensity (mV^2/s); the average power per second, during the event
F. Peak-to-peak amplitude (mV); the maximum amplitude – minimum amplitude, observed during the event
G. Classification; arbitrary number system (enumeration) designed by Michael Chang
    - 0: unclassified event, these events typically have artifacts or are contaminate by low-level noise. Require human intuition to remove from analysis or keep.
    - 1: Seizure-like Event (SLE)
    - 1.5: Questionable SLE (smaller seizure, may require human intuition to determine if SLE
    - 2: Interictal Event (IIE)
    - 2.5: Questionable IIE, not yet sure if it's a IIE or a collection of spikes (IISs)
    - 3: Interictal spike (IIS), or a collection of IIS
        - Regardless, events that are classified as such (3) will be returned to the recording to be detected again using a wavelet transform
    - 4: Artifact: these are events that have been contaminated by noise
H. Light-triggered; indicate if event was triggered by a external stimulus (1), or if it was spontaneously initiated (0)
I. Results of the classification system for Spike Rate;
    - 1, if event was above threshold
    - 0, if event was below threshold.
    - Number in the top column indicates the threshold, determined by the algorithm.
J. Results of the classification system for Intensity;
    - 1, if event was above threshold
    - 0, if event was below threshold.
    - Number in the top column indicates the threshold, determined by the algorithm.
K. Results of the classification system for Duration;
    - 1, if event was above threshold
    - 0, if event was below threshold.
    - Number in the top column indicates the threshold, determined by the algorithm.
L. Results of the detection algorithm for Artifacts (based on peak-to-peak amplitude);
    - 1, if event was above threshold
    - 0, if event was below threshold.
    - Number in the top column indicates the threshold, determined by the algorithm.
M. Tonic Phase; the tonic phase was defined as 2 continuous seconds of frequency that is at least 1/3 maximum frequency during the event (floor set at 1 Hz).

- 1, if tonic phase is present
- 0, if tonic phase is absent

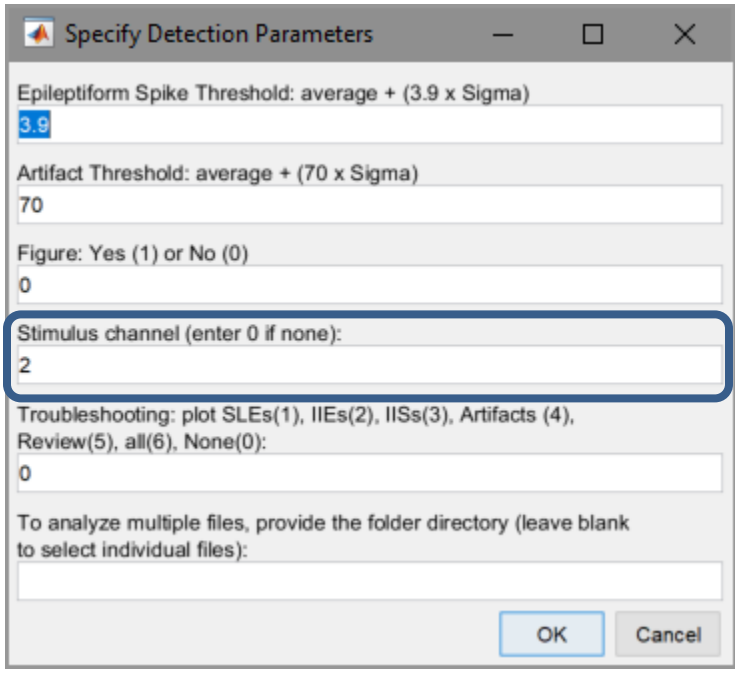
N. Average Preictal Frequency; number is only valid if tonic phase is present (Column M = 1)
O. Average Tonic Frequency; number is only valid if tonic phase is present (Column M = 1)
P. Average Clonic Frequency; number is only valid if tonic phase is present (Column M = 1)
Q. Minimum Frequency observed during the tonic phase, if tonic phase is present (Column M = 1)
R. Intensity Ratio, time period of high intensity divided by time period of low intensity, during the epiletiform event
S. Minimum intensity ratio minimum to be considered a SLE
    - 1, if event was above threshold
    - 0, if event was below threshold.
    - Number in the top column indicates the threshold, determined by the algorithm.
T. Minimum intensity ratio minimum to be considered an IIE
    - 1, if event was above threshold
    - 0, if event was below threshold.
    - Number in the top column indicates the threshold, determined by the algorithm.
U. [N/A]
V. Start Time (Tonic Phase); number is only valid if tonic phase is present (Column M = 1)
W. End Time (Tonic Phase); number is only valid if tonic phase is present (Column M = 1)
X. Label, determined by a human (Michael)
    - 0: unclassified event, these events typically have artifacts or are contaminate by low-level noise. Require human intuition to remove from analysis or keep.
    - 1: Seizure-like Event (SLE)
    - 1.5: Questionable SLE (smaller seizure, may require human intuition to determine if SLE
    - 2: Interictal Event (IIE)
    - 2.5: Questionable IIE, not yet sure if it's a IIE or a collection of spikes (IISs)
    - 3: Interictal spike (IIS), or a collection of IIS
        i. Regardless, events that are classified as such (3) will be returned to the recording to be detected again using a wavelet transform
    - 4: Artifact: these are events that have been contaminated by noise
Y. Michael's comments about the event based on the .pptx file

A.3 Tips for running the Epileptiform Detection Algorithm

1. Filter the data (LFP signal, not the stimulus signal) before analysing it with the algorithm
    a. 60 Hz notch filter
    b. Electrical Interference:
        i. Harmonic from 1 to 5
        ii. Cycles to average 10
        iii. Reference Frequency 60 hz



2. If your computer 'runs out of memory'
    a. Restart your computer and run again
    b. Double check you entered the correct stimulus channel (enter 0 if none)



    c. Split your data into smaller chunks and analyze separately.

Appendix B Defining epileptiform events in acute seizure models

A continuum of electrographic signals, referred to as epileptiform-like events, manifested in rodent brain tissue after treatment with proconvulsant perfusion media (i.e. 4-AP and Zero-Mg^{+2}). These epileptiform-like events were classified into two major categories: ictal-like events and interictal-like events, based on guidance from the literature (Anderson et al., 1986; Avoli and de Curtis, 2011; de Curtis and Gnatkovsky, 2009; Dulla et al., 2018; Fisher et al., 2014; Lillis et al., 2012; Traub et al., 1996) and supervision by a team of clinically practicing medical doctors specializing in epilepsy and medical researchers studying epilepsy models at the Toronto Western Hospital (Chang et al., 2018; Chang et al., 2019).

The following are the rules for demarcating ictal events based on literature and domain knowledge of the *in vitro* 4-AP seizure model, programmed into this epileptiform event detection algorithm.

B.1 Defining ictal-like events

Ictal-like events, also known as seizure-like events, are electrographic signals that manifest in rodent brain tissue after treatment with proconvulsant perfusion media (i.e. 4-AP [100 uM]). These ictal-like events are reminiscent of the electrographic signals observed in the intracranial electroencephalography (iEEG)-recordings of *in vivo* animals and humans during a seizure episode. According to the literature, ictal-like events are defined based on the following characteristics:

- **Amplitude** is at least 4x the standard deviation (sigma) of the baseline signal
    - Amplitude of the ictal-like spiking activity was at least four times the baseline's sigma (Zhang et al., 2019)
- **Duration** is at least 5 s long
    - The duration to differentiate ictal events from interictal events is subjective and vary between seizure models
    - In general, ictal events are longer than interictal events
    - A duration of at least 10 s is used clinically (Abend and Wusthoff, 2012)
    - Although rare, clinical seizure as short as 2 s has been observed (Devinsky et al., 1988)

    - Electrographic seizures are described to have a duration of at least 5 s when observed in *in vitro* seizure models (Lillis et al., 2012) and i*n vivo* epilepsy models (Smith et al., 2018)
    - In this study, the threshold value for ictal event duration is set to be 5 s, as it was the average between commonly used thresholds
- **Tonic-like firing phase** is present, and last for at least 2 s
    - The Tonic-like firing phase is high frequency population spiking activity that is at least 1 Hz
    - The tonic-like firing phase corresponds with the tonic phase of a seizure observed in intact animals (Anderson et al., 1986)
    - The tonic-like firing phase was arbitrarily required to last at least 2 seconds, as that was the shortest time a seizure has been clinically recorded (Devinsky et al., 1988)
- **Onset** is demarcated to be the start of the preictal spike, also known as the "sentinel spike" (Appendix B-1)
    - The preictal spikes are large amplitude spikes that precede the fast ictal spiking activity (de Curtis and Gnatkovsky, 2009)
    - Although these spikes are labelled "preictal", they are consistently observed at the very onset of a ictal event (and clinical seizures) and considered to be an integral part of the ictal event (Fisher et al., 2014)
- **Offset** is demarcated to be the last spike of the ictal-like event
    - The offset as the point where ictal activity has returned to baseline activity (<4x the baseline sigma)

B.2 Defining interictal-like events

Interictal-like events are all the epileptiform events that occur between two ictal events. In other words, they are all the epileptiform-like events that cannot be classified as an ictal-like event. According to that definition, there can be a great variety of interictal-like events that all look very different. There are four major subcategories of interictal-like events:

i. **Interictal-like spikes** (IISs)

- A single deflection in the LFP recording

ii. **Spike and wave events**

- A single deflection in the LFP recording followed by small oscillations

iii. **Stereotypical interictal-like events** (IIEs)

- A disperse collection of epileptiform-like spikes that lack the necessary features or thresholds to be considered an ictal-like event
- IIEs typically have a duration below the threshold to be classified as an ictal-like event or lack a tonic-like firing phase

iv. **Bursting activity**

- Short duration high frequency activity that is <2 s
- These high frequency bursting activities are spread apart by >1 s

B.3 Rules for demarcating epileptiform events in *in vitro* 4-AP cortical seizure model

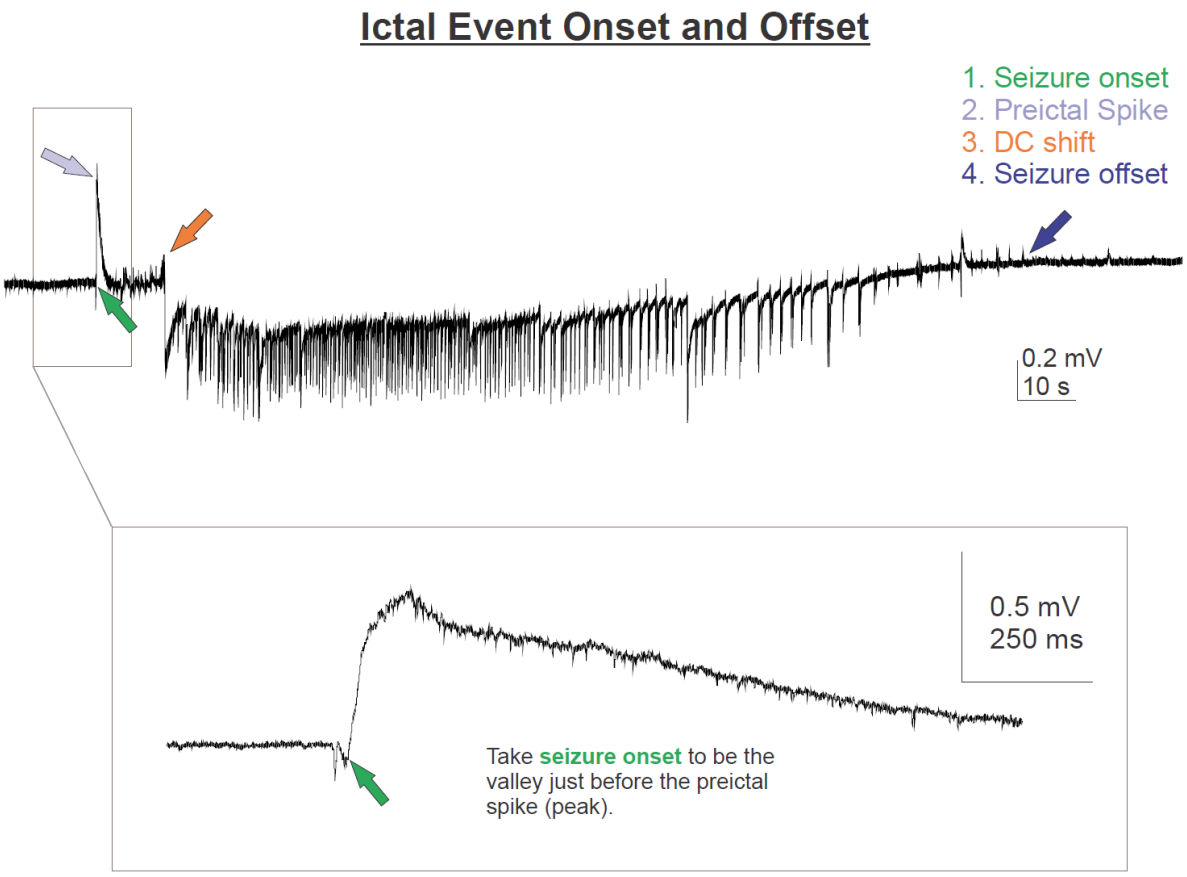
B.3.1 Epileptiform event onset defined as (which ever phenomenon is observed first):

1. Preictal Spike
    - The onset is demarcated as the valley just before peak (Appendix B-1, green arrow)
    - Typically, followed by increased ripples and a DC shift
2. DC Shift
    - The onset is demarcated as the point just before the drop (Appendix B-1, orange arrow)
3. Oscillations
    - if there is no spike or DC shift observed
    - The onset is demarcated as the point that oscillations begin

B.3.2 Epileptiform event offset defined as (which ever phenomenon is observed first):

1. Oscillations end (Appendix B-1, blue arrow)
2. Recording returns to baseline, for at least 10 s
3. Poly-spikes cease

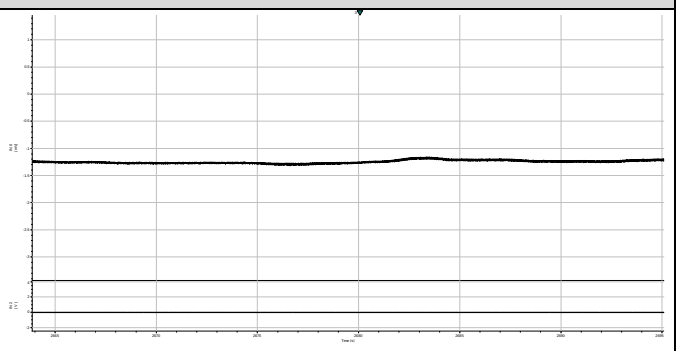
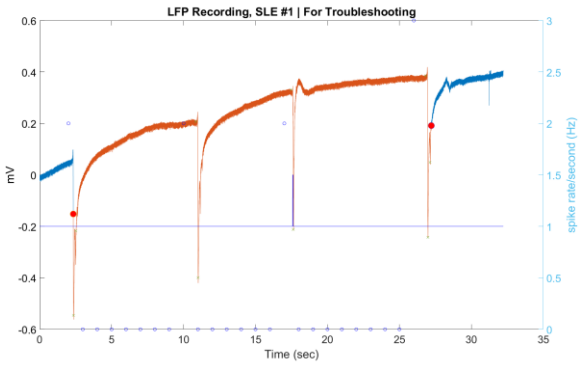
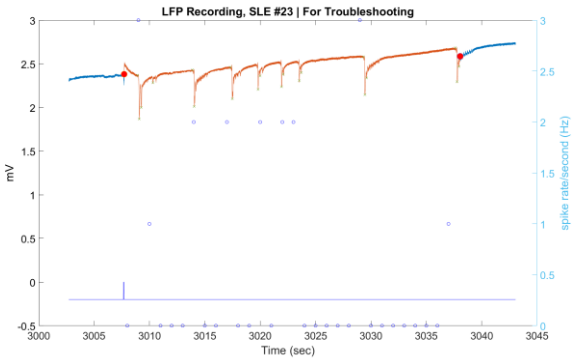
   Note: If last spike is due to light stimulus, disregard

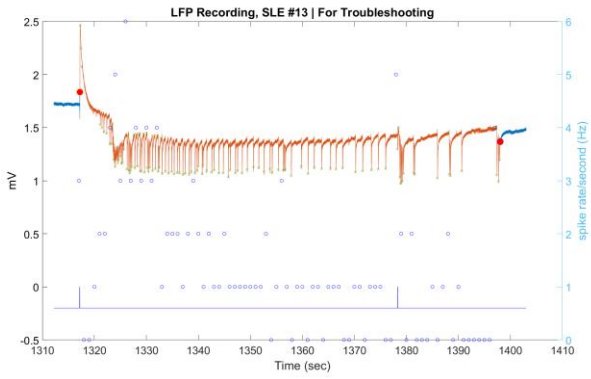
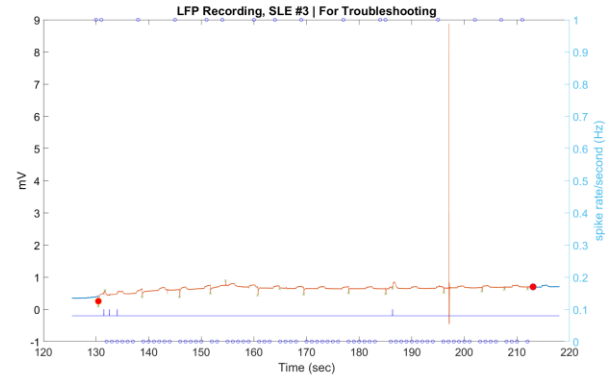


Appendix B-1: Prototypical ictal-like event (electrographic seizure-like event) recorded from *in vitro* 4-AP cortical seizure model. Zoomed-in view of the preictal spike (inset) shows the exact point of seizure onset (green arrow).

B.4 Six possible events that may occur in the *in vitro* 4-AP seizure model

Appendix B-2: Commonly observed events in *in vitro* 4-AP cortical seizure model, unfiltered extracellular LFP recordings.

Event	Description	LFP recording
Interictal period (baseline)	Flat line, no activity	
Interictal Spike(s)	Sharp spike(s) with a narrow width ~100-250 ms and high amplitude. The maximum physiological amplitude is never greater than 70x the standard deviation of the baseline.	
Interictal Event	A collection of spikes that are sparse and spread out (low frequency)	

Ictal Event	A collection of high amplitude spikes occurring at a high frequency. A defining feature is the “high frequency” phase (>5 Hz) that lasts >2 s	
Artifact	A high amplitude spike that is narrow in width >10 ms, and can have unlimited amplitude (saturate the recording limits)	
Noise	Repetitive oscillations that are obtuse shaped. They can occur when the bath application of perfusion media causes the recording electrode to move