ON THE IDENTIFICATION AND CHARACTERIZATION OF EPOCHS OF SIGNIFICANT CHANGES IN FOCAL NEOCORTICAL SEIZURES

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ABSTRACT

Epilepsy affects 2-3% of the population. To study the emergence and spread of focally initiated seizures, we used the 4-aminopyridine (4-AP) model, a well-established, reliable model of acute focal neocortical seizures. It is important to understand what changes in the cortical circuit allow a highly-correlated firing state to emerge, evolve, and recur after focal-cortical injury. Using the calcium indicator GCaMP6 with in vivo 2-photon cellular microscopy, we examined the activity profiles of individual neurons in layer 2/3 (L2/3) of the visual cortex during focal seizures induced following intracortical 4-AP injections. Here we propose two methodologies for detecting the epochs of significant activity in the context of focal seizures induced following intracortical 4-AP injections: a novel methodology based on the noise-interval identification of the fluorescence signal of *each neuron* and another one based on the recurrence quantification analysis (RQA), a powerful tool based on the topological analysis of the phase space of the underlying dynamics. They provide a methodology for accurately identifying the onset of significant events, dissecting the mechanisms of seizure initiation and propagation within the field of view, and highlighting the recruitment of neurons in various regions of the field of view.

Index Terms— focal neocortical seizures, epilepsy, recurrence quantification analysis, temporal correlation

1. INTRODUCTION

Epilepsy is a group of neurological disorders affecting 2-3% of the global population. Seizures involve the generation and propagation of hyper-synchronous activity across cortical circuits. Brain injury patients carry high risk of epilepsy for decades following injury [1], causing morbidity. Injury causing epilepsy typically leads to excitation/inhibition imbalance, which drives neural circuits into self-perpetuating oscillatory activity states, feeding the hyper-synchronous epileptic bursts seen on cortical-surface EEG [2, 3]. Our knowledge about how neurons interact to generate the abnormal network activity patterns that underlie ictogenesis is limited [4] and it is critical to understand what changes in the cortical circuit allow a highly correlated firing state to emerge, evolve, and recur after focal-cortical injury. Here we employ the 4-aminopyridine (4-AP) model, a well-established, reliable, model of acute focal neocortical seizures.

The underlying dynamical system that governs the behavior of a neuron can be very complex, with its dynamical features being partially observed via the corresponding recorded signals (e.g. fluorescence). Furthermore, the relevant physiological phenomena are typically a fusion of deterministic, chaotic, and random processes, yielding changes at multiple time scales, which imposes significant challenges on the subsequent signal analysis.

A critical question is whether the use of statistical features of the ongoing neuronal firing enables us to accurately detect the ictal events and signify the occurrence of certain internal states. Despite the significant progress, the automatic detection of these events, as well as of their dynamic changes, is still an open problem. This work proposes two methodologies for detecting the epochs of significant activity in the context of focal seizures induced following intracortical 4-AP injections, namely, (a) a novel methodology based on the noise-interval identification of the calcium signal of each neuron, and (b) a method based on the recurrence quantification analysis (RQA) [5, 6] of the recorded signals, for the detection of critical transitions in the underlying time-evolving dynamics.

The above two methods are applied on the fluorescence signals from one mouse during epileptic seizures, and comparatively analyse their outcome. The main results are consistent in terms of the number of the events that were identified, their start, and their duration. The noise-interval based technique applies simple statistics on the df/f signal of *each neuron* and serves as "baseline"/benchmark technique. The RQA is applied on the *population* df/f signal, i.e., summed all the df/f across all neurons in the field of view (FoV), and thus captures a more "macroscopic" view of the behaviour.

The rest of the paper is organized as follows: Section 2 describes the data acquisition and preprocessing approaches, whilst Section 3 discusses the most recent related work. Sec-

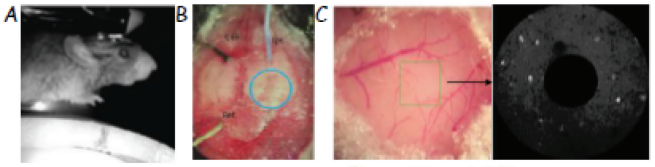

Fig. 1. A. The mouse is headposted rested awake and free to walk on a treadmill. B. Skull picture showing electrode implantation and site of planned window to overly visual cortex. C. (Left) Craniotomy window after implantation. Green frame illustrates approximately the field of view (FOV). (Right) FOV (diameter $\approx 500\mu m$) showing spontaneous activity scanned by the spiral scanning method.

tion 4 analyzes the two methodologies proposed for detecting epochs of significant activity, followed by a comparative performance analysis. Then, Section 5 discusses our observations about recruitment of neurons. Finally, Section 6 summarizes the main outcomes and gives directions for further extensions.

2. DATA ACQUISITION AND PREPROCESSING

Experiment was performed on an adult (>8 weeks old) Thy1-GaMP6s (C57BL/6J-Tg (Thy1-GCaMP6s) GP4.3Dkim/J) mouse which expresses the GCaMP6s calcium indicator in pyramidal neurons [7]. Two-photon calcium imaging was performed using an in vivo Ultima multiphoton microscope (Bruker, Madison, WI) equipped with a Ti:Sapphire Insight pulsed laser (Sprectra Physics, Santa Clara, CA). Calcium signals were imaged through a 16x water immersion lens (Nikon, Tokyo, Japan; 0.8 NA, 3 mm working distance) by exciting the genetically encoded calcium indicator at 920 nm. The field of view (FoV) was scanned in spiral mode at approximately 5 Hz. We imaged layer 2/3 pyramidal neurons at $\sim$ 150 μm from the pial surface. A 150-200 μl solution of 12.5 μM 4-AP or an equal volume of vehicle (0.9% NaCl) was injected 1 mm antero-laterally to the imaged FoV at the level of the primary visual cortex (V1) at the infragranular layer ($\sim$ 600 μm deep from the pial surface).

About 20 min after each injection, the post injection activity was recorded. Each recording lasts approximately 10 min. We have three different measurements for the same mouse: pre-injection (control), then post-vehicle injection, and post-4-AP injection. Here we report our preliminary analysis for the post 4-AP injection using the df/f of the signal, which corresponds to the raw fluorescence signal minus the baseline of the signal over the baseline, where the baseline corresponds to the 10^{th} percentile of the raw signal.

3. RELATED WORK

Cortical injections of chemoconvulsants are thought to induce a focus of epileptiform activity from where seizures propagate in the surrounding network [8]. Epileptiform activity in this surrounding network (often referred to as propagation area) have been shown to engage cortical neurons in a spatio-temporal ordered fashion [9], suggesting that neurons that are physically closer to the injection site get recruited earlier than neurons located at a larger physical distance. In a follow-up study [10], the recruitment of neurons at the level of the injection site was compared with the recruitment of neurons in the propagation area. In such study, the recruitment of large proportion of neurons in the initiation site appeared to occur simultaneously (at the temporal resolution of the recordings), suggesting that the engagement of neurons in the injection site might not only be determined by their physical distance. Using wide-field calcium imaging, Rossi *et al.* [11] have shown that also brain areas at larger distances to the initiation site might get recruited earlier than areas physically closer to it depending on their homotopic connections to the injection site areas. Whether similar rules govern recruitment in the injection area as well is to be determined.

RQA has been recently applied to EEG data from epilepsy patients as well as from epileptic rats, obtaining encouraging preliminary results in feature detection and identification of normal, interictal, and ictal EEG signals [12, 13, 14].

4. IDENTIFICATION OF SIGNIFICANT ACTIVITY

This section describes in detail our two approaches for detecting epochs of significant activity, namely, one based on the noise intervals of the df/f signal of each neuron and the RQA-based method applied on the population df/f signal, i.e., summing the df/f signal across all neurons of the FoV. The RQA-based method applies the global RP on the population signals (i.e., aggregate signal of neurons over the FoV). The methodology is generic and applicable on other datasets and neuronal populations.

Noise-interval based identification We define as *local* plateau of a neuron an epoch of *significantly* high calcium activity. The start and end of the plateaus of each neuron is identified based on the noise intervals of the df/f signal of the neuron. Specifically, we estimate the Gaussian distribution that consists of the df/f values less than the 20^{th} percentile of the neuron's df/f, "mirroring" also the right part. We estimate the standard deviation and define as noise intervals all the frames that are less than the mean plus two standard deviations of this synthetic distribution. We ignore the small noise intervals of duration under a certain threshold. Moreover, we merge events with small inter-arrival intervals between consecutive noise intervals. The noise intervals that remain correspond to the "valleys" and the in-between periods indicate the "plateaus", which are epochs of significant activity.

The *global* plateaus correspond to significant activity of the neuronal population in the FoV and are identified as follows: If the population/aggregate time series that indicates the number of neurons which have their own local plateaus

at each frame is above a certain threshold, then the global plateaus are marked.

RQA based identification A key component of the proposed pipeline is the recurrence plot (RP), a square matrix whose elements express when a state of a dynamical system recurs, thus revealing all the timestamps the *phase space trajectory* of the dynamical system visits roughly the same area in the phase space. To this end, RPs enable the investigation of an m-dimensional phase space trajectory through a 2-D representation of its recurrences. Such recurrence of a state occurring at time i, at a different time j is represented within a 2-D square matrix with ones (recurrence) and zeros (non-recurrence), with both axes corresponding to time.

Given the population df/f of length N, $\{r_i\}_{i=1}^{N}$, a phase space trajectory is reconstructed via time-delay embedding, $\mathbf{x}_i = [r_i, r_{i+\tau}, \ldots, r_{i+(m-1)\tau}]$, $i = 1, \ldots, N_s$ where m is the embedding dimension, τ is the delay, and $N_s = N - (m-1)\tau$ is the number of states. Having constructed a phase space representation, the RP is defined by, $\mathbf{R}_{i,j} = \Theta\left(\varepsilon - \|\mathbf{x}_i - \mathbf{x}_j\|_p\right)$, $i, j = 1, \ldots, N_s$, where $\mathbf{x}_i$, $\mathbf{x}_j \in \mathbb{R}^{\,m}$ are the states, ε is a threshold, $\|\cdot\|_p$ denotes a general ℓ_p norm (Euclidean distance ($p = 2$) is commonly used), and $\Theta(\cdot)$ is the Heaviside step function, whose discrete form is defined by

$$\Theta(n) = \begin{cases} 1, & \text{if } n \geq 0 \\ 0, & \text{if } n < 0 \end{cases} , \; n \in \mathbb{R} \, . \tag{1}$$

The resulting matrix $\mathbf{R}$ exhibits always a main diagonal, $\mathbf{R}_{i,i} = 1$, $i = 1, \ldots, N$, also known as the *line of identity* (LOI). A major advantage of RPs is that they can also be applied to rather *short* and even *non-stationary* data.

Since we are interested in detecting precisely the onset and offset times of seizure events in the associated sequence $\mathbf{t}$, a global RP can enhance the understanding of the phase space trajectories and detect *phase synchronous* dynamics even when two distinct states of $\mathbf{r}$ do not converge.

Estimation of embedding parameters. In our implementation, the optimal time delay τ is estimated as the first minimum of the average mutual information (AMI) function [15]. Concerning the embedding dimension m, a minimal sufficient value is estimated using the method of false nearest neighbours (FNN) [16]. In practice, the minimal embedding dimension is defined as the dimension for which the fraction of false neighboring points is zero, or at least sufficiently small.

State-change onset/offset detection method. A key property of RPs, which is exploited in the detection of state-change instants, is that it reveals the local difference of the dynamical evolution of close trajectory segments in the phase space of the population df/f signal. A time dilation or a compression of the time intervals, where a state-change appears in the population df/f, causes a distortion of the diagonal lines in the corresponding RP. Then, the LOI will be disrupted yielding the, so called, *line of synchronization* (LOS) [5]. Although the LOS is continuous, it is not a straight diagonal line. This enables the estimation of a *non-parametric rescaling function* between the states of the population df/f.

Let $\mathbf{l} \in \mathbb{R}^{N_s}$ denote the LOS. The interpretation of $\mathbf{l}$ is the following: if $l_i = k$, for some $i = 1, \ldots, N_s$, then, the state of the the population df/f at time i approximates the state at time k. In the case of the population df/f, the LOS is a piecewise linear function. Since, in general, $N_s \neq N$, in practice we apply a zero padding to $\mathbf{r}$ in order to obtain a LOS vector $\mathbf{l}$ whose length is equal to that of the index vector $\mathbf{t}$.

Finally, having estimated the LOS, we calculate the first-order differences, $d_{l,i} = l_{i+1} - l_i$, $i = 2, \ldots, N$. Doing so, the vector $\mathbf{d}_l \in \mathbb{R}^N$ will be of the form, $\mathbf{d} = [\ldots, \ldots, 0, d_i, 0, 0, \ldots, 0, d_j, 0, \ldots]$, with the zeros corresponding to the intervals where the LOS is constant. Then, given that $d_i \neq 0$ and $d_j \neq 0$, we consider d_i to be the onset time and d_j the offset time of an epoch of significant activity. This interpretation is justified by the fact that the constant segments of the LOS, or equivalently the zero segments of $\mathbf{d}_l$, correspond to time periods in the population df/f whose dynamics, as expressed by the corresponding state vectors, are driven by the same seizure.

Consistent results between RQA and noise-interval based method in the identification of the epochs of significant activities. The identification of the epochs of significant activity based on the noise-interval approach (plateaus) and the RQA events is shown in Fig. 3 (top). After the merging of the small RQA events, according to the same procedure as for global plateaus and valleys, 16 events remain (marked with orange color), whilst the noise-interval-based identification results in 14 events (marked with green color). The duration of these events varies (Fig. 3 (bottom)). A sensitivity analysis was performed to define the thresholds of the short noise intervals and their inter-arrival as well as the definition of global plateaus in terms of percent of simultaneous local plateaus. Our main findings qualitatively remain consistent across different thresholds. We are in the process of validating our findings with traces collected from several other mice. The start and end of events set by the RQA events tend to be earlier and later, respectively, compared with the start and end of the global plateaus identified by the noise-interval-based method. This can be attributed to the time-delayed embedding process, which generates state vectors whose elements may span adjacent regions of the signal with different dynamics. Doing so, RQA is able to "foresee" upcoming switching regimes with respect to the inherent dynamics, when entering ("onset" times) these regions, while still maintaining some memory when exiting ("offset" times) them.

5. RECRUITMENT OF NEURONS

For each neuron, and for each global plateau, the relative time lag of its onset of its local plateau compared to the corresponding global plateau onset is estimated. Negative lag

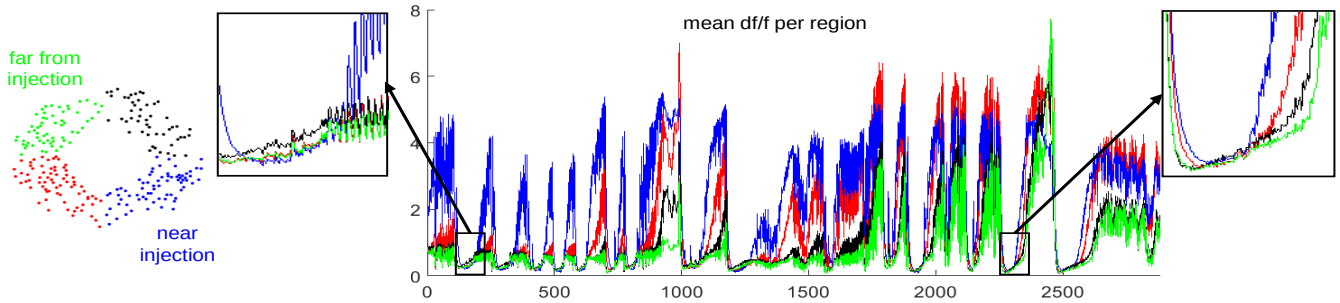

Fig. 2. Mean df/f of the subpopulations of neurons at the four regions of FoV neurons (colored based on their distances from the injection point). Two representative global plateaus are zoomed to better examine the mean df/f of each region.

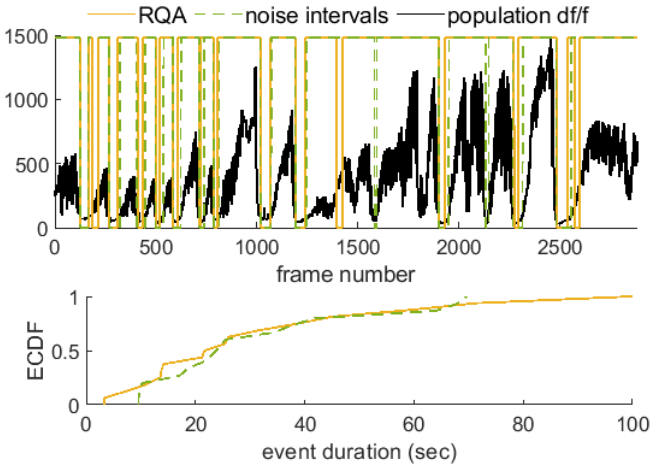

Fig. 3. Events identified by RQA (gold color) and noise interval method (green color) (top). Comparative ECDFs of the event duration (bottom).

means that the neuron starts prior to the global onset, while positive means the neuron is following after the global onset. We also estimate for each neuron the percentages of its local plateaus that precede, are in sync, and follow the global plateaus onsets. The majority of neurons are recruited after the onset of the global plateau. Neurons, with onsets before the start of global plateaus, are spread all over the FoV, suggesting that the effect of 4-AP is long-range (consistent with Rossi *et al.*'s finding [11]). Taking into consideration the level of the df/f as well as the lags of neurons from different regions with respect to the approximate injection point, our analysis revealed two phases of the ictogenesis process: In the first phase, up to about the frame 1500 ($\sim$ 5min), the sub-population near the injection point (blue color in Fig. 2) reaches relatively high df/f levels, while the other three sub-populations have significantly lower values. During that phase, the "blue" population has larger lag compared to the other sub-populations, with respect to the global plateaus onsets. As the epileptic activity evolves, the sub-population near the injection point starts its ictal events earlier than the others (on average) (Fig. 4 (right)) and the df/f of the other populations increases.

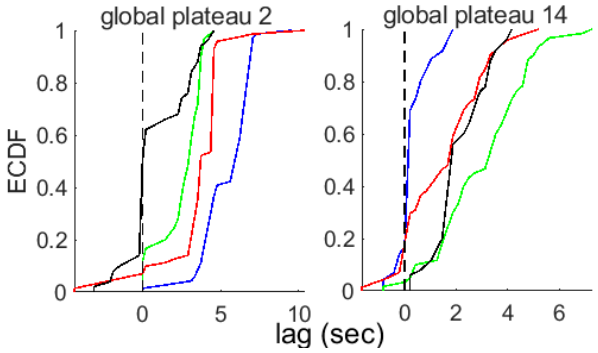

Fig. 4. ECDFs of the lag of the local plateau of each neuron with respect to the onset of the global plateaus 2 and 14, (left) and (right), respectively; colored based on their region Fig 2, left.

6. CONCLUSIONS AND FUTURE WORK

It is the first time that such advanced RQA-based techniques have been applied to identify the neuronal network patterns of interest, and dissect, in vivo, the mechanisms of focal epilepsy. We are in the process of validating our results with datasets from other mice. Our long-term objective is to inform the mechanisms of seizure initiation and propagation to more precisely model in vivo epileptic cortical networks. We aim to examine the finer spatio-temporal dynamics of the ictogenesis process, and micro-phases, including the order of the recruitment of neurons in the seizures, integrating information from the EEG. We plan to examine whether the EEG signal can reveal information about the states of other neurons (e.g., interneurons outside of the FoV) that may play important role in the propagation or in the control of seizures. The use of LSTM is one direction that we will pursue. They have been recently applied in EEG data to declare an imminent seizure and can nicely integrate the temporal dimension and the sequence that identified neuronal patterns manifest during the evolution of ictal-events.

7. REFERENCES

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