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Synchronization analysis of voltage-sensitive dye imaging during focal seizures in the rat neocortex

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Seizures are often assumed to result from an excess of synchronized neural activity. However, various recent studies have suggested that this is not necessarily the case. We investigate synchronization during focal neocortical seizures induced by injection of 4-aminopyridine (4AP) in the rat neocortex \textit{in vivo}. Neocortical activity is monitored by field potential recording and by the fluorescence of the voltage-sensitive dye RH-1691. After removal of artifacts, the voltage-sensitive dye (VSD) signal is analyzed using the nonlinear dynamics-based technique of stochastic phase synchronization in order to determine the degree of synchronization within the neocortex during the development and spread of each seizure event. Results show a large, statistically significant increase in synchronization during seizure activity. Synchrony is typically greater between closer pixel pairs during a seizure event; the entire seizure region is synchronized almost exactly in phase. This study represents, to our knowledge, the first application of synchronization analysis methods to mammalian VSD imaging \textit{in vivo}. Our observations indicate a clear increase in synchronization in this model of focal neocortical seizures across a large area of the neocortex; a sharp increase in synchronization during seizure events was observed in all 37 seizures imaged. The results are consistent with a recent computational study which simulates the effect of 4AP in a neocortical neuron model. © 2011 American Institute of Physics. [doi:10.1063/1.3640043]

INTRODUCTION

Traditionally, researchers and clinicians have assumed that epileptic seizures are characterized by excess synchronized electrical activity in the brain (Wong et al., 1986). This view goes back at least as far as the seminal work of Penfield et al. (1954). The widespread acceptance of the view that “epilepsy is synchrony” continues to be seen in studies which attempt to localize seizure onset zones in human patients based on increased synchrony (Dauwels et al., 2009), attempt to predict seizures based on synchrony (James and Gupta, 2009; Mirowski et al., 2009), or use deep brain stimulation techniques to modulate synchrony in order to alleviate seizures (Fine et al., 2009).

To some extent, the assumption that synchrony correlates with seizures arises from the tacit assumption that increases in oscillating field potential or electroencephalography (EEG) amplitude must be provoked by an increase in synchronization, and therefore, it is often concluded that the large regular oscillations observed in seizures imply an increase in synchrony (Steriade, 2003; Niedermeyer, 2005; see discussion in Uhlhaas and Singer, 2006). Other studies have investigated synchronization during seizures quantitatively, using analysis techniques based on nonlinear dynamics and information theory. Among these studies, the range of observations has been diverse. Van Putten (2003) found increases in nearest-neighbor electroencephalographic synchrony in human subjects with temporal lobe epilepsy and generalized seizures. Ben-Jacob et al. (2007) observed an increase in synchrony in electrocorticography (ECoG) signals during seizures in human subjects. Their results indicated a good correspondence...
between the overlap of the highly synchronized area determined by their method with the area removed in surgery and success of the surgery. A recent in vitro hippocampal study showed “a massive, hypersynchronous cluster” of high frequency (>100 Hz) oscillations in CA1 coinciding with the onset of seizure activity (Jiruska et al., 2010).

In contrast, other studies report either a decrease in synchrony during seizure events or other results inconsistent with the “hypersynchronous” view of epileptic seizures. Netoff and Schiff (2002) observed a decrease in synchrony between pairs of neurons during seizure activity in vitro, using whole-cell patch clamp recording in hippocampal slices. Navarro et al. (2007) demonstrated a decrease in synchrony between all EEG electrodes, one of which was located at the seizure focus, in a rat model of status epilepticus. Kramer et al. (2008) used a linear coupling measure between electrode pairs in human EEG recordings to construct a network of inter-electrode dependencies, finding a “diffuse breakdown in global coupling” at seizure onset. Van Drongelen et al. (2003) suggest, based on a study in mouse neocortical slices, that increased electrical activity during elicited seizure-like events is caused by neural recruitment, rather than an increase in synchrony.

Other studies have produced ambiguous results. A magnetoencephalography (MEG) study in human subjects with generalized seizures found an increase in local synchrony but also, in some cases, observed a drop in synchronizaton between distant brain regions (Garcia Dominguez et al., 2007). Wendling et al. (2003) found a drop in spatial correlation between stereoelectroencephalographic electrodes in high frequency activity in human subjects at seizure onset, which was followed by low frequency, high amplitude activity showing an increase in synchrony. Lai et al. (2007), applying dynamical techniques to analyze human EEG and ECoG recordings, observed that “[w]hile there are cases where the overall degree of synchronizaton tends to increase during the seizure, there are relatively more cases where synchronization decreases.” They remark that their result “means that future monitoring and possibly therapeutic techniques for epileptic seizures based on synchronization are likely to be highly individualized.”

An additional motivation to revisit fundamental assumptions regarding synchrony during seizures is provided by recent studies which show a decrease in synchrony immediately prior to seizure onset in EEG recordings from human patients (Le Van Quyen et al., 2001, 2003; Mormann et al., 2003). This observation relates not only to the investigations of seizure mechanisms but also to the tantalizing problem of whether dynamical methodologies can be used for the prediction of seizures, a hope which, if realized, would obviously be of immense clinical usefulness (Lehnertz et al., 2007; Mormann et al., 2007; Lehnerz, 2008). However, Joupy et al. (2005) observed no pre-ictal increase in synchrony, but rather a pronounced post-ictal rise in synchrony in human EEG recordings.

Here, we use voltage-sensitive dyes to image spatiotemporal patterns of synchronization during focal seizures in the rat neocortex. We observe a rapid, statistically significant increase in synchronization during seizure events.

METHODS

Surgery and staining

All procedures used were approved by the University of Missouri at St. Louis’s Institutional Animal Care and Use Committee (IACUC). Adult male Sprague-Dawley rats (241–573 g) were initially anesthetized with intraperitoneal ketamine (90 mg/kg) and xylazine (4.0 mg/kg) and then sustained with urethane (1.0–1.5 g/kg). Temperature was maintained at 37°C with a heating blanket (Harvard Apparatus, Holliston, MA). Heart rate was monitored (SurgiVet, Waukesha WI) and maintained stable. The electrocardiogram (EKG) was monitored between leads attached to the right hindlimb and the skin of the scalp. The rat was ventilated with room air via an animal respirator (Kent Scientific, Torrington, CT). The recorded EKG and respiration rate were used subsequently for removal of heartbeat and breathing artifacts from the recorded data (Lippert et al., 2007; see below for more detail).

The rat was placed in a stereotaxic frame, the skin above the skull shaved, and the scalp incised along the midline. A cranial window (~5 mm by 9 mm) was made over one hemisphere, between lambda and bregma (Fig. 1), and the dura was removed for dye staining. A solution of the voltage-sensitive dye RH-1691 (Optical Imaging, Inc., 1.0–2.0 mg/ml in 1% NaCl solution) was applied to the cortex for 1–2 h. After staining, the cortex was washed with a 1% NaCl solution for 15 min.

Electrophysiology

For local field potential (LFP) recording, a glass micro-pipette filled with 1% NaCl was positioned approximately 400 μm below the cortical surface, with its tip in contact with cortical layers II-III. The field potential and EKG were recorded using a DBA-S system (World Precision Instruments, Sarasota, FL), digitized at 2000 Hz by a CED Power 1401 (Cambridge Electronic Design, Cambridge, UK), and recorded onto a PC using Spike 2 software.

Drug administration

The injection of 4AP was performed using the method of Yang et al. (2002). A glass pipette, filled with a solution...
of 4AP (25 mM in 1% NaCl), was attached to a Nanoproject II ooocyte injector (Drummond Scientific, Broomall, PA) and positioned less than 1 mm from the field potential electrode (see Fig. 1). A total volume of 0.5 μl of the 4AP solution was injected approximately into cortical layers II-III, in increments of 50 nl. Typically, multiple focal seizures occurred following the initial injection; when no seizure occurred for more than 20–30 min, an additional set of injections of 4AP was performed.

**Imaging**

Agar (1.5% in 1% NaCl) and a glass cover slip were placed over the cortex, which was illuminated using a 100 W tungsten-halogen bulb; light was passed through a microscope and a 630 ± 20 nm interference filter (Optical Imaging, Inc.) and reflected down onto the cortex via a dichroic mirror (Optical Imaging, Inc.). The fluorescence of the dye from the stained cortex was filtered by a 695 nm long-pass filter (Optical Imaging, Inc.) and recorded by a 16-bit CCD camera (Cascade 512E, Princeton Instruments), placed over a tandem configuration of two 50 mm lenses (Ratzlaff and Grinvald, 1991). The camera was focused 300–500 μm below the cortical surface in order to de-emphasize the changes in reflectance from the surface blood vessels in preference to the neocortical layers. Images were typically recorded at a frequency of 70 Hz and were synchronized with the recorded field potential via a transistor-transistor logic (TTL) pulse. After binning, each pixel represented an area of 96 μm by 96 μm.

**Noise and artifact reduction**

The recording time of voltage-sensitive dye imaging experiments is limited by dye bleaching (Jin et al., 2002). In the case of in vivo imaging, dye may also be washed away due to blood circulation (Lippert et al., 2007). These factors cause a drift in the baseline fluorescence intensity. In the present study, each imaging run typically lasted about 15 min, and about 10 imaging runs were performed for each experiment. Due to the extended recording times necessary in order to image full seizure events from onset to termination, the effects of dye bleaching were a serious consideration. For the data presented here, baseline drift was removed using regression by a 10th order polynomial.

In addition to baseline drift, a large oscillation at very low frequency (<0.2 Hz) was observed in the VSD signal. This low frequency activity may result from some combination of electrophysiological activity (low frequency peaks are sometimes seen in LFP recordings), vasomotor activity possibly confused with intrinsic optical signal, or possibly camera drift. There was no clear correlation between the low-frequency activity and seizure events in the present study. Therefore, we have performed the analysis described below after removing this low-frequency component with high-pass filtering at 0.2 Hz. Random fluctuations were also observed in the recorded VSD signal which are presumed to result from the statistical fluctuation of the number of photons arriving at the CCD (i.e., photon or shot noise) and by the process of quantifying the electronic signal on the CCD (i.e., read noise). This noise was reduced by spatial averaging over 3 by 3 neighboring pixels at each frame. This is reasonable, assuming that noise of this kind is statistically independent (or uncorrelated) between signals from different CCD sensors.

The fluorescent dye used in the present experiments is RH-1691, a “blue dye” specifically developed to reduce contamination due to hemodynamic noise since it is excited with a longer wavelength than the absorption wavelength of hemoglobin (Shoham et al., 1999; Civillico and Contreras, 2005). The use of such “blue dyes” is particularly important in seizure studies, where each seizure is a unique event, and thus imaging trials cannot be averaged in order to improve the signal-to-noise ratio, as is commonly done in multi-trial somatosensory studies. Despite the use of a blue dye, however, hemodynamic artifacts cannot be completely eradicated, and we observed a sharp peak in the VSD signal power spectrum at a frequency corresponding to the heartbeat. Lippert et al. (2007) have suggested that this might be caused by physical movement (e.g., movement of blood vessels caused by the heartbeat) rather than absorption of light by hemoglobin. To reduce this heartbeat artifact, a template of the artifact was constructed by triggered averaging of the voltage-sensitive dye signal with reference to the EKG signal as suggested by Ma et al. (2004) and Lippert et al. (2007).

After removing the dura, movement of the brain surface synchronized with the respiration of the rat was often clearly visible, even to the naked eye. Corresponding periodic oscillations at the respiration frequency were observed in the voltage-sensitive dye signals. This artifact was removed by a triggered averaging procedure similar to that used for removal of heartbeat artifact. Averaging was triggered by electrical pulses received from the ventilator, corresponding to the timing of the animal’s respirations. Figure 2 shows VSD signals during a seizure before and after removal of the noise and artifacts described above.

![FIG. 2.](Image) (a) Raw VSD signal, plotted as percent change with respect to the average intensity over the time interval shown, before removal of artifact. (b) VSD signal, again shown as percent change with respect to average intensity, after signal processing as described in Methods. (c) Local field potential (LFP) recording from the same location and time interval as the VSD signals shown in panels (a) and (b). Note the correspondence between the peaks in the LFP signal and the peaks in the VSD signal shown in panel (b), after the removal of artifacts and noise.
Determination of seizure area

After seizure onset was recorded from the field potential electrode, it was determined whether a given pixel, in a given time window, should be included in the seizure area by comparing the power spectrum of the VSD signal at that pixel during the seizure event to the corresponding pre-seizure power spectrum (Yang et al., 2002). The power spectrum was calculated using a sliding window of 1000 data points (about 10–13 s) with a 100 point shift. For each time window, the sum of the five largest power spectrum peak values in the frequency range of 1–10 Hz was determined. This frequency range was chosen based on the observation that the peaks in the power spectrum during seizure events appear in this frequency range. In each time window, a pixel was classified as part of the seizure area if the sum of the five largest peak values exceeded a threshold value determined from the pre-seizure state. The threshold value was set at 1.5 times the maximum of the summed peak values from sliding time windows in the pre-seizure state. A representative example of seizure areas is shown in Fig. 3.

Synchronization analysis

Stochastic phase synchronization analysis was applied to the voltage-sensitive dye signals. This technique is appropriate in cases where the amplitudes of two time series are uncorrelated, but the signals nonetheless maintain an approximately fixed phase relationship (Pikovsky et al., 2003). After noise and artifact reduction, the time course of the fluorescence intensity from each pixel was filtered using a zero-phase bandpass digital filter (Butterworth 4th order, 1–10 Hz). Phase synchronization analysis was performed, using a sliding window of 1000 data points (about 10–13 s) with a 100 point shift. For a given data set, a pixel which was estimated to be at the location of 4AP injection was chosen as a “reference” pixel. For each sliding window, the synchronization index was calculated for every pixel with respect to the reference pixel. All the analysis was performed using custom-written programs in MATLAB (The Mathworks).

The instantaneous phase \( \phi(t) \) of the VSD signal was extracted by applying a Hilbert transform in order to construct the analytic signal

\[
\zeta(t) = x(t) + ix_H(t) = A(t)e^{i\phi(t)}
\]

where \( x_H(t) \) is the Hilbert transform of the original signal \( x(t) \),

\[
x_H(t) = \frac{1}{\pi} P.V. \int_{-\infty}^{\infty} \frac{x(t)}{t-\tau} d\tau
\]

and \( P.V. \) indicates the Cauchy principal value (Rosenblum et al., 1996).

The phase difference between two signals is calculated as the difference between their instantaneous phases. To evaluate the degree of synchronization, a histogram of the phase differences is made within a time window. The synchronization index, which corresponds to the intensity of the first Fourier mode of the probability density of the phase difference, is calculated as

\[
\gamma = \sqrt{(\cos \Delta \phi(t_i))^2 + (\sin \Delta \phi(t_i))^2}
\]

where \( \Delta \phi(t_i) \) is the phase difference at the \( i \)th time point in a time window and the brackets represent the time average over the window (Rosenblum et al., 1996). The synchronization index ranges from 0 to 1; \( \gamma = 1 \) if two signals are perfectly synchronized (i.e., \( \Delta \phi \) is constant in time), while \( \gamma = 0 \) if two signals are not synchronized at all (\( \Delta \phi \) is uniformly distributed).

RESULTS

Field potential activity during seizures

Voltage-sensitive dye imaging was successfully obtained for a total of 37 seizures from 3 rats. The first seizure after the initial 4AP injection usually occurred within a few minutes following the injection. Seizures occurred multiple times without additional injection of 4AP. While seizure duration varied both within the same rat and among rats, seizures tended to be short (about 10–60 s) immediately after injection of 4AP and to become longer, eventually reaching a duration of 200–300 s, without additional injection of 4AP. Typical inter-seizure intervals were 1–3 min, though they tended to increase (up to 20–30 min) at later stages of an

\[\text{FIG. 3. (Color online) Time evolution of seizure area. Left: Patterns of seizure area in the neocortex. The white pixels are classified as belonging to the seizure area using the criterion described in the main text. The red number in each picture represents time in seconds, corresponding to the time scale of the plots shown on the right. Scale bar indicates ~ 1 mm. Top right: LFP as a function of time, recorded from a location near the site of 4AP injection. Bottom right: Seizure area in mm}^2\text{ as a function of time.} \]
experiments. Voltage-sensitive dye imaging data sets which consisted of both a whole seizure event and a pre-ictal period of more than 15 s were used for further analysis.

While the pattern of field potential activity varied from one rat to another, and indeed from one seizure to another within a single animal, seizure events typically began with a large field potential spike followed by relatively fast oscillations with increasing amplitude. In the middle of a seizure event, periodic spike-and-wave activity with constant large amplitude was often observed. Toward the end of each seizure, firing typically became irregular and bursting patterns were often observed.

Analysis of the field potential recordings consistently showed a dominance of power below 10 Hz, though the power spectrum did exhibit changes throughout the duration of each seizure, and from one seizure to another. The dominance of frequencies below 10 Hz led to the restriction of the current study to an investigation of synchronization in the 1–10 Hz range; future work may be extended to investigate synchronization of activity with frequencies >10 Hz.

**Change of seizure area over time**

An example of the changes in seizure area, determined by the method described above, is shown in Fig. 3. The epileptic area typically increased quickly at the beginning of a seizure event and remained approximately constant throughout the duration of the seizure. A sudden decrease of the area is observed at the end of each seizure. In the particular example shown, the area drops sharply around 200 s and stays almost constant from 200 to 250 s, while the field potential amplitude increases, and begins to display an irregular bursting pattern. It is possible that the drop in seizure area may correlate with the transition of the field potential from spike-and-wave activity to bursting activity, but further experiments will be necessary in order to support this hypothesis.

**Phase synchronization**

An example of the changes in synchronization indices over time from multiple pairs of pixels throughout the seizure area is shown in Fig. 4. As seen from this example, a dramatic increase in synchronization is observed at seizure onset. One can also observe that closer pairs of pixels show stronger synchronization than pairs separated by a larger distance. Even widely separated pairs of pixels within the seizure area, however, show a significant increase in synchronization during the seizure event. (Note that the spatial scale of even the local synchrony shown here is much greater than that of the correlations introduced by the spatial averaging performed in order to eliminate random noise; thus, the synchronization we observe is not introduced by smoothing of the data.)

To investigate the time course of synchronization behavior over the entire imaged area of the neocortex, rather than just between selected pixel pairs, the synchronization index was determined for every pixel in each image, with respect to a reference pixel located adjacent to the 4AP injection site. This allows the visualization of synchronization maps, shown in Figs. 5 and 6 for two different seizures. As time increases after seizure onset, the synchronized region increases in both area and degree of synchronization. In the middle of seizure, most of the pixels in the recorded area are well synchronized with the 4AP injection site. At seizure termination, the synchronized region decreases in both area and degree of synchronization. Note that individual seizure events exhibit individual, idiosyncratic characteristics: for example, the seizure shown on the left hand side of Fig. 5 begins as a single synchronized area, while that depicted in

---

**FIG. 4.** (Color) **Lower panels** show LFP and synchronization index $\gamma$ over the time course of a seizure event; **top panels** show the seizure area, determined as described in Methods, at corresponding times during the course of the seizure. Scale bar shows ~1 mm. The synchronization index $\gamma$ is shown as a function of time, for the pixel pairs corresponding to the colored lines in the seizure maps. The pixel pairs chosen always included one pixel approximately at the 4AP injection site.
Fig. 6 begins with two separate areas, one enclosing the injection site and the other distant from it. As the seizure progresses, the two areas coalesce.

In order to quantify the overall synchronization, an averaged synchronization index $\bar{\gamma}$ was calculated by averaging the synchronization indices with respect to the reference pixel, in each sliding window. The bottom right panels in Figs. 5 and 6 show $\bar{\gamma}$ as a function of time for the two representative seizures; the corresponding field potential recording near the 4AP injection site is shown in the top right panel of each figure. A dramatic increase in $\bar{\gamma}$ was observed during seizure events.

In order to perform a statistical analysis, a paired t-test was performed for all 37 seizure events, in order to compare the maximum pre-seizure synchronization index $\bar{\gamma}$ with that during the seizure (Fig. 7), giving $p < 0.00001$. Thus, the maximum $\bar{\gamma}$ during seizure events was significantly larger than $\bar{\gamma}$ preceding the seizures.

Phase maps

The synchronization index, as defined here, provides a measure of whether the oscillating VSD signals maintain a (relatively) constant phase difference over time. It does not provide information about whether the phases of any pair of signals maintain a constant in-phase relationship and whether they are consistently anti-phase (i.e., one signal at its minimum while the other is at its maximum) or exhibit some intermediate behavior. In order to examine the relative phases of the VSD oscillations, we investigated the phase itself, determined at each pixel from the Hilbert transform described above. In Fig. 8, we show spatial maps of the phase value at each pixel. Note that, in contrast to the synchronization index, the phase is not measured “with respect to” anything else, but it is a characteristic of the VSD time signal at each pixel. The distribution of the phase over space at a given time shows a clear difference between the periods:

Fig. 5. (Color) Left panels: spatial maps of synchronization index with respect to a reference pixel, at various time points during the seizure. Number shown in each panel corresponds to time points on the horizontal axis of the right panels. Color scale shows synchronization index value; scale bar indicates ~1 mm. Top right panel: local field potential recording near the site of 4AP injection, over the time course of the seizure. Bottom right panel: spatially averaged synchronization index ($\bar{\gamma}$) on the same time scale as the field potential. (For vertical dotted red line, see Discussion section.)

Fig. 6. (Color) Similar to Fig. 5, but showing a seizure event in another rat. Note the two distinct areas of high synchronization early in the seizure (33–44 s).
before and during the seizure event. Before the seizure event, the phase distribution looks almost random. On the other hand, during the seizure event, most of the pixels show an in-phase pattern, although it is often observed that one region is slightly advanced in phase with respect to the rest of the seizure area.

The degree of the concentration of the distribution of the phase at a given time can be evaluated by the following measure (Tass, 1999):

\[
R(t) = \left| \frac{1}{N} \sum_{k=1}^{N} \exp[i\phi_k(t)] \right|
\]  

(4)

where \(\phi_k(t)\) is the phase of the signal from the \(k\)th pixel at time \(t\) and \(N\) is the total number of pixels in a two-dimensional image. A value of \(R(t) = 1\) indicates perfect in-phase synchronization. The example shown in Fig. 8 indicates a clear increase in \(R(t)\) during the seizure event, with predominantly in-phase activity.

Discussion

The results presented here demonstrate a sharp increase in synchronization during seizure events in all 37 seizures imaged. Moreover, we observe a pattern of in-phase electrical activity, as illustrated in Fig. 8. This result is consistent with a recent computational study which simulates the effect of 4AP in a neocortical neuron model (Takeshita et al., 2007).

When interpreting the results of this study in the light of the disparate observations of synchrony, and the lack thereof, reported in the literature, it is important to note that differences in the results might be caused by the systems studied. For example, Netoff and Schiff (2002) studied synchrony using whole-cell patch recording from a pair of pyramidal neurons in hippocampal slices. Their recording technique measures electrical activity at a single neuron level, while the voltage-sensitive dye imaging performed here measures activity at a population level. Furthermore, while the results in Netoff and Schiff (2002) were obtained using 4AP-induced seizures, as are the results shown here, 4AP was applied globally in to an in vitro preparation, in contrast to the local injection applied here.

Garcia Dominguez et al. (2005) applied phase synchronization analysis to MEG recordings from human patients with generalized seizures. Their results show that, while local synchrony (between neighboring pairs with distance less than 4 cm) increased during seizures, distant synchrony (>4 cm) sometimes decreased. They commented about this lower synchrony that “although not uncommon, this marked desynchronization between particular channels during the ictal [seizure] period was not the rule in the patients studied.” The Garcia Dominguez et al. study concerns generalized seizures in human subjects, in contrast to the focal seizures imaged here.

FIG. 7. Maximum \(\bar{\gamma}\) before and during seizures, averaged over the 37 seizures imaged. Error bars show standard deviation.

FIG. 8. (Color) Left panels: spatial maps of phase at various time points during the seizure. Number shown in each panel corresponds to time points on the horizontal axis of the right panels. Color scale shows phase value; scale bar indicates \(\sim 1\) mm. Top right panel: local field potential recording near the site of 4AP injection, over the time course of the seizure. Bottom right panel: \(R(t)\), a measure of the distribution of phases, is shown on the same time scale as the field potential.
Lai et al. (2007) developed a new measure for the overall synchrony among multivariate signals based on a random matrix criterion and applied the measure to signals from EEG and ECoG recordings from epileptic patients. The authors reported relatively more cases where the overall synchrony decreased during seizures. Again, it should be emphasized that there is no a priori reason why human absence seizures and partial seizures with secondary generalization, as studied by Lai et al., should have the same synchronization characteristics as focal neocortical seizures in rats.

In studies with epileptic patients, it has been reported that there is a dependence of the degree of synchrony on the frequency band of signals studied (Garcia Dominguez et al., 2005). The frequency range of epileptic activity investigated in the present study (1-10 Hz) is much narrower than that the 3-55 Hz range investigated by Garcia Dominguez et al. It is possible that increased synchrony may occur during seizures in certain frequency ranges and not in others, and that this too may underlie some of the disparate results within the literature.

Another problem for further investigation is the correlation between synchrony and spatiotemporal evolution of the seizure event. For example, one could ask whether there are differences in synchrony during different time stages of the seizure event. In the present study, the synchronization index was calculated for pairs of pixels, one of which was always located at the 4AP injection site. However, synchrony with respect to a pixel distant from the injection site may reveal different patterns of synchrony, including a more variable structure throughout the development of the seizure event.

As described above, the local field potential recording during seizures shows a variety of firing patterns such as periodic spiking and bursting. Another subject for further investigation is the relationship between various field potential oscillation patterns, such as spike-and-wave, low voltage fast activity, or bursting, and synchrony. It is also possible that the degree of synchrony may correlate with local field potential amplitude. Note, for example, in Fig. 5, there is a slight decrease in the averaged synchronization index around 70 s. When the synchronization index is at its local minimum, the local field potential recording undergoes a sudden decrease in amplitude (see dotted red line in Fig. 5). A mechanism for such a correlation, however, remains to be proposed.

In addition to possible correlations with field potential oscillation patterns or amplitude, it should also be investigated whether increases in synchrony correlate with increases in the spatial extent of the seizure. In this context, it is interesting to note that our preliminary results indicate a very strong correlation between the seizure area defined using the criterion outlined above and illustrated in Fig. 3, and the seizure area defined by a criterion based on a minimum threshold value of γ.

Finally, it should be noted that our experiments suggest that the presence of the voltage-sensitive dye itself alters the dynamics of the seizure event. Seizures induced by 4AP injection without the presence of the dye occur much more frequently than when neocortex is stained with RH-1691 (data not shown). This may be due to phototoxic effects of RH-1691 on the neural activity (Jin et al., 2002). A recent study has shown that some voltage-sensitive dyes, including the one used in the present study, affect the function of GABA-A receptors (Mennerick et al., 2010). While we observed that the occurrence of seizure events was less frequent in VSD-stained cortex, no difference was observed in field potential recordings of seizures in stained vs. unstained tissue. Moreover, no difference in phase synchronization was observed between seizures measured at the beginning of an experiment compared to those recorded at the end of an experiment. Nevertheless, the possible disturbance of the system by the probe itself obviously warrants some caution in the interpretation of the results.

Our findings demonstrate a significant increase in the overall synchrony during focal neocortical seizures in the rat neocortex. Synchrony was greater between closer pixel pairs during a seizure event. The entire “epileptic” region is synchronized almost in phase. These results strongly indicate that the picture of seizure activity as characterized by a high degree of synchronous neural activity cannot be discounted. However, in light of the other studies discussed above, which indicate drops in synchrony in other seizure models, the possibility must be considered that some forms of seizure activity may exhibit increases in synchrony, while others may show the opposite. A major challenge facing the field is to develop testable models for seizure dynamics which encompass these disparate results. A recent computational study showing a mechanism by which an initial decrease in phase coherence at seizure onset may be followed by an increase in phase coherence after seizure initiation (Li et al., 2008) may begin to point the way toward an understanding of these complex phenomena. The road from in silico models to the human brain, however, will undoubtedly be a long one.

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Huygens, C., Horologium Oscilatorium (Parisis, France, 1673).


