Development of hypersynchrony in the cortical network during chemoconvulsant-induced epileptic seizures in vivo

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Cymerblit-Sabba A, Schiller Y. Development of hypersynchrony in the cortical network during chemoconvulsant-induced epileptic seizures in vivo. J Neurophysiol 107: 1718–1730, 2012. First published December 21, 2011; doi:10.1152/jn.00327.2011.—The prevailing view of epileptic seizures is that they are caused by increased hypersynchronous activity in the cortical network. However, this view is based mostly on electroencephalography (EEG) recordings that do not directly monitor neuronal synchronization of action potential firing. In this study, we used multielectrode single-unit recordings from the hippocampus to investigate firing of individual CA1 neurons and directly monitor synchronization of action potential firing between neurons during the different ictal phases of chemoconvulsant-induced epileptic seizures in vivo. During the early phase of seizures manifesting as low-amplitude rhythmic β-electrocorticography (ECoG) activity, the firing frequency of most neurons markedly increased. To our surprise, the average overall neuronal synchronization as measured by the cross-correlation function was reduced compared with control conditions with ~60% of neuronal pairs showing no significant correlated firing. However, correlated firing was not uniform and a minority of neuronal pairs showed a high degree of correlated firing. Moreover, during the early phase of seizures, correlated firing between 9.8 ± 5.1% of all stably recorded pairs increased compared with control conditions. As seizures progressed and high-frequency ECoG polyspikes developed, the firing frequency of neurons further increased and enhanced correlated firing was observed between virtually all neuronal pairs. These findings indicated that epileptic seizures represented a hyperactive state with widespread increase in action potential firing. Hypersynchrony also characterized seizures. However, it initially developed in a small subset of neurons and gradually spread to involve the entire cortical network only in the later more intense ictal phases.

 METHODS

Animals and surgery. All experiments were performed on 4- to 6-wk-old male Wistar rats as previously described (Cymerblit-Sabba and Schiller 2010). The rats were anesthetized with intraperitoneal urethane (10 ml/kg of 20% urethane dissolved in normal saline). After the rat was anesthetized, lidocaine (2%) was locally injected into the scalp region. Later, the rats were held with a short metal rod glued to the posterior aspect of the skull with dental cement. The skin covering the skull was opened, and the skull was exposed. A small craniotomy was drilled through the skull 4.8 mm posterior to bregma and 3.6 mm lateral to the midline using a dental drill, and the dura covering the cortex was carefully removed using fine forceps. Throughout all experiments, body temperature was maintained at 37.5°C using a closed-loop animal blanket system (FHC), and the response to pain and spontaneous whisking was monitored. Additional urethane was applied if rats developed spontaneous whisking or response to pain. In the “awake rat experiments,” rats were first anesthetized with the gas anesthesia halothane, and lidocaine (2%) was locally injected. While the rats were under this anesthesia, the craniotomy was performed and either subdural ECoG or intrahippocampal multielectrode bundles were placed in the hippocampus and fixated to the skull using dental cement (for details see Seizure induction). After the electrodes were firmly fixated, halothane was discontinued and the rats woke up. Before discontinuation of halothane, additional lidocaine was injected locally to avoid logical signals, which sum the electrical activity from thousands of neurons and millions of synapses and filter out fast local events such as action potentials (Creutzfeld et al. 1966; Nunez and Srinivasan 1981). As a result, EEG and ECoG recordings cannot reliably monitor network synchronization at the single neuron firing level.

To reliably evaluate synchrony in a network, one must record and compare the timing of action potential firing between different neurons (Salinas and Sejnowski 2001). Surprisingly, up to date we have little data on neuronal firing and degree of neuronal synchronization based on direct recordings of action potential firing of single neurons during seizures in vivo. Moreover, data from in vitro brain slice studies reported conflicting results regarding neuronal synchronization during seizures (Netoff and Schiff 2002; Cohen et al. 2006). In this study, we directly addressed these issues. We simultaneously recorded the local ECoG and single-unit and multunit activity from intrahippocampal multielectrode bundles to characterize action potential firing of individual neurons and examine the degree of neuronal synchronization of action potential firing during the different ictal phases of chemoconvulsant-induced epileptic seizures in vivo.

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EEG and ECoG recordings are relatively gross electrophysiol...
pain at the craniotomy site. All experiments were approved by the Technical Institutional Animal Ethics Committee.

Seizure induction. We used three different methods to evoke seizures: 1) Systemic application of pilocarpine: pilocarpine (300 mg/kg) dissolved in normal saline was injected intraperitoneally. 2) Local intrahippocampal application of kainate: 0.5 µl of kainate (2 µg/µl dissolved in artificial cerebrospinal fluid) was injected into the CA3 region of the hippocampus. 3) Local intrahippocampal application of a pilocarpine, kainate, and picrotoxin mix: 0.5 µl of a chemoconvulsant mix composed of pilocarpine (100 mM), picrotoxin (0.2 mM), and kainic acid (2 µg/µl) dissolved in artificial cerebrospinal fluid was injected into the CA3 region of the hippocampus. Local intrahippocampal application of the chemoconvulsants (either kainate or the pilocarpine, kainate, and picrotoxin mix) was performed by local injection into the CA3 hippocampal subfield (3.14 mm posterior to the Bregma, 2.6 mm lateral to the midline, and depth of ~3.6 mm) using a metal cannula and a syringe pump (WPI, SP100IZ). In local-induced seizures, chemoconvulsants were applied to the CA3 rather than the CA1 hippocampal subfields for two main regions. First, application of chemoconvulsants into the CA1 region was much less efficient in evoking seizures. Second, injections in close proximity to our recording electrode markedly compromised the quality of the recordings.

We chose to use three models of epilepsy. Systemic pilocarpine-induced seizures evoked recurrent discrete seizures that evolved in many cases to intense polyspikes ictal activity, while local intrahippocampal-induced seizures had a known site of origin. Regarding the focal models, we locally administered a triple convulsant mixture (kainate/pilocarpine/picrotoxin) in addition to isolated kainate, as the triple mixture was more efficient in inducing seizures and typically evoked more intense ictal activity.

At the end of the experiment, the site of injection was confirmed histologically (for details, see Electrophysiological recordings). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) except for kainate that was purchased from Tocris Biosense.

For our unit, analysis during seizures we only analyzed three seizures per rat. We chose the first three seizures that developed the third most intense ictal phase (high-amplitude polyspikes with or without accompanying slow waves). In case there were less than three seizures that developed the third ictal phase, we chose the earliest seizures instead. Moreover, we excluded from our analysis segments in which seizure activity was limited to some of the electrodes or when seizure activity was different between the different electrodes.

Electrophysiological recordings. In general, all recordings were performed as previously described (Cynoberblit-Sabba and Schiller 2010). A bundle of five tungsten metal electrodes was inserted in the CA1 region of the hippocampus (4.8 mm posterior to the Bregma, 3.6 mm lateral to the midline, and depth of ~2.3–2.8 mm). The reference electrode was placed in the epidural space over the contralateral frontal region. The electrode bundle was lowered to the CA1 hippocampal subfield using high precision stereotactic micromanipulator (TSE Systems). The depth of the CA1 region was determined electrophysiologically. Typically, when the electrodes passed the neocortex single-unit activity was recorded. Later, as the electrodes passed through the subcortical white matter and stratum oriens of the hippocampus, no activity was recorded. When the electrodes reached the cell body layer of the CA1 region, single-unit activity was again recorded. At the end of each experiment, the location of the recording electrodes was examined histologically.

The electrode bundles we used for the experiments were composed of five individual insulated tungsten electrodes (diameter of 200 µm, sharpened at the tip to 2–5 µm) with an impedance of 1–2 MΩ. To obtain the electrode bundle, the five individual electrodes were glued in a rectangular configuration with one line containing three electrodes and the other line containing two electrodes (We-Sense). The interchange distance between neighboring electrodes was 0.2–0.3 mm, and the entire electrode bundle had a width of 0.4–0.6 mm and length of 0.6–0.9 mm. Typically, we obtained reliable recordings from four to five of the electrodes. In addition, in some experiments one to two silver wires were implanted subdurally over the frontal hemispheres bilaterally, and the ground silver electrode was inserted subdurally over the anterior contralateral frontal region.

During the recordings, the recorded signals were amplified (X × 1,000), filtered (0.1–10,000 Hz), and stored in a computer using the ME-16 amplifier and MR-Crack software (MEA, Germany). The digital sampling rate of our recordings was 25 KHz.

To confirm the location of the recording electrodes after the experiments were completed, the brains were removed, sliced, and examined histologically. At the end of the experiments, we either removed the brain and sliced it acutely (200- to 300-µm thick) using a vibrotome as previously described or removed the brain after heart perfusion first with saline and then with 4% paraformaldehyde in 0.1 M phosphate buffer. Later, the fixed brains were sliced (50- to 200-µm thick) and stained with Nissel staining.

In a separate set of experiments, we performed EEG and ECoG recordings in behaving rats. In these experiments, four stainless steel wires were inserted into the CA1 region of the right hippocampus and in the epidual space above the right and left frontal regions.

Analysis. Analysis of the data was performed for the most part offline. After completion of the experiments, the recorded data were replayed and filtered with two different band-pass filters: 0.5–100 Hz to obtain the local EEG/ECoG and 1–5 KHz to obtain spikes. The spike data were later sorted into single-unit activity according to a combined threshold and shape detector. Sorting was performed with the MC-Rack software (MEA) and the off-line spike sorter (OS-3) by Plexon. Due to the high action potential firing rates during seizures, we only sorted spikes from one to two neurons in each electrode for each sorting run. To obtain the spike trains of all neurons recorded by the electrode, we repeated the runs off-line twice while placing the threshold at positive and negative values.

We carefully performed the sorting of spikes including histograms of the interspike intervals (ISI) and excluded uncertain neurons from our analysis. It is important to stress that during some segments of seizures single-unit activity could not be reliably sorted, especially during the third most intense ictal phase of seizures. These segments were excluded from analysis. To overcome movement artifacts and artifacts caused by the fact some epileptiform spikes are not fully filtered out by the 1- to 5-KHz band path, we first simultaneously observed the data filtered at 1–5 KHz and 1–100 Hz. In case movement artifacts or artifacts caused by epileptiform spikes showed on the spike trace (filtered at 1–5 KHz), this segment was excluded. During the first and second ictal phase of systemic-induced seizures and the first ictal phase of local-induced seizures, we excluded only small segments of the recordings (<5%). During the second ictal phase of local-induced seizures, we excluded 11.4 ± 2.0% of the seizure duration (n = 7), and during the third ictal phase, we excluded 35.3 ± 5.2% (n = 7) of the seizure duration. In awake rats, we were only able to analyze 47.1 ± 15.4% of the seizure time.

The spike trains obtained with the MC-Rack software were exported to the NeuroExplorer software (Nex Technologies), which allowed us to calculate the firing rates of individual neurons, the pair-wise cross-correlation function, the probability for pair-wise simultaneous firing, and the statistical significance levels. Further analysis of the data and plotting of graphs were performed with the Igor software (WaveMetrics).

RESULTS

To characterize neuronal firing and synchronization of action potential firing between different neurons during chemoconvulsant-induced seizures, we performed simultaneous recordings from a bundle of five metal microelectrodes inserted into the CA1 region of the hippocampus. From the recordings of each electrode, we extracted both the local ECoG and the
single-unit activity by using different band pass filters and sorting the unit data to different neurons (for details see METHODS). Chemoconvulsant-induced seizures were evoked either by systemic (ip) application of pilocarpine (300 mg/kg) or by local intrahippocampal application (0.5 μl) of either kainate (2 μg/μl) or a chemoconvulsant mix composed of kainate (2 μg/μl), pilocarpine (100 mM), and picrotoxin (0.2 mM). Systemic application of pilocarpine resulted in recurrent seizures (Fig. 1A). On average seizures, these seizures lasted 29.5 ± 18.8 s and the average frequency of seizures was a seizure every 160.1 ± 29.4 s (0.00624 Hz; 89 seizures in 10 rats; means ± SD). In contrast to systemic-induced seizures, local intrahippocampal application of the chemoconvulsants resulted in prolonged continuous seizure activity.

The first step of our analysis was to characterize the different ictal ECoG patterns developing during chemoconvulsant-induced epileptic seizures. In our experiments, seizures were induced either by systemic (ip) application of pilocarpine.

Similar to human seizures, the ictal ECoG activity of chemoconvulsant-induced seizures evolved as seizures progressed. In general, the seizures we observed were composed of three different phases. In seizures induced by systemic pilocarpine, the initial ictal phase was composed of low-amplitude rhythmic β- or β-γ-activity (25.3 ± 7.0 Hz in 26 rats). This rhythmic β-activity appeared either continuously or as recurrent bursts lasting 0.5–1 s (Fig. 1B). The second ictal phase consisted of periodic low-frequency (0.5–2 Hz) sharp waves that were usually accompanied by bursts of rhythmic β-activity (Fig. 1C). The third and most intense ictal phase consisted of high-amplitude polyspikes with or without accompanying slow waves (Fig. 1D). It is interesting to note that similar to our seizures many human partial seizures initiated with low-amplitude rhythmic β-activity (for review see, de Curtis and Gnatkovsky 2009) and that polyspikes frequently developed during the later phases of human partial seizures (Fisch 1999; Kaplan and Tatum 2008).

In general, seizures gradually progressed from the first to the third ictal phase. However, not all seizures developed all three ictal phases. While low-amplitude rhythmic β-activity was observed in almost all seizures and all rats, periodic sharp waves were only observed in 31% of seizures and 50% of rats and polyspikes developed in 42% of seizures and 69% of rats. Typically, the third ictal phase (polyspikes with or without slow waves) was followed by transient seizure termination.

Behaviorally in anesthetized rats seizures manifested as convulsions in the face and limbs. In some cases, convulsions were observed already during the initial low-amplitude rhythmic β-ictal phase, while the third polyspikes ictal phase was usually accompanied by more intense convulsions. As rats were anesthetized during seizures, we could not determine the effect of seizures on their state of consciousness. To further study the correlation between the electrographic and clinical manifestations, we also performed EEG and ECoG recordings in awake behaving rats (n = 4) in systemic pilocarpine-induced seizures. We observed that rhythmic β-activity on the ECoG could be accompanied by nose wiping and head shaking, probably representing partial seizures, while during polyspike activity (3rd ictal phase) generalized convulsions were presented.

Seizures induced by local intrahippocampal application of either kainate or a mixture of pilocarpine, kainate, and picrotoxin were also composed of three ictal phases (Fig. 2). Similar to systemic pilocarpine the initial ictal phase of seizures induced by local chemoconvulsants consisted of low-amplitude rhythmic β- or β-γ-activity (average peak frequency of 27.4 ± 9.2 Hz in 21 rats; Fig. 2). The second ictal phase of these seizures consisted of rhythmic high-frequency spikes (average frequency of 25.6 ± 7.9 Hz, observed in 17 of the 21 rats recorded; Fig. 2). In some rats (6 out of 21 rats), a third more intense ictal state developed. This more intense ictal phase resembled the third ictal phase induced by systemic pilocarpine and consisted of recurrent discharges of polyspikes followed by slow waves (Fig. 2). Seizures induced by intrahippocampal kainate (5 rats) and a mixture of pilocarpine, kainate, and...
During the third polyspikes ictal phase. During the initial low-amplitude rhythmic β-ictal phase, the average firing rates reached values of 438 ± 69 and 278 ± 36% of the control values in systemic (304 neurons in 26 rats) and local (245 neurons in 21 rats)-induced seizures. During the third polyspikes ictal phase, the average firing rates further increased and reached values of 731 ± 92 and 702 ± 81% of the control values in systemic- and local-induced seizures (Fig. 4). Although the average firing rates of action potentials markedly increased during seizures, this did not occur in all neurons (Fig. 4, B and F). During the initial low-amplitude rhythmic β-ictal phase, the firing rates increased significantly in ~70% of neurons, while ~95% of neurons significantly increased their firing rates during the more intense third polyspike ictal phase (a significant increase in the firing rate was defined as a significant increase in the average firing rate compared with control conditions with a P significance level that exceeded 0.05). In the remaining neurons, the firing frequency of action potentials either did not significantly change or even decreased (Fig. 4, B and F).

The increased firing frequency of action potentials also manifested as a reduction of the ISI during both systemic- and local-induced seizures (Fig. 4, C, D, G, and H). Both the average mean and median ISI gradually decreased as ictal phases progressed. For example, the median ISI decreased by 48.1 and 81.5% compared with control values during the first and third ictal phases of systemic pilocarpine-induced seizures, (Fig. 4C).

During the initial ictal phase in seizures evoked by systemic pilocarpine, rhythmic β-activity was observed intermittently (Fig. 1). We compared the firing rates of neurons during segments with and without the rhythmic β-ECOG activity. Surprisingly, there was only a small albeit significant difference between the two segments. When the ECOG showed rhythmic β-activity, the average firing rate was increased to 489 ± 58% of the control values compared with 413 ± 41% of the control value when no accompanying rhythmic β-activity was observed on the ECOG (P < 0.01, 304 neurons in 26 rats).

Synchronization of action potential firing between different neurons during the different ictal phases of seizures. The prevailing view is that hypersynchrony lies at the “pathophysiological heart” of epileptic seizures (Fisher et al. 2005; Engel and Pedley 2008). However, this dogma is based for the most part on the EEG and ECOG rather than on direct action potential recordings. We next characterized synchronization of action potential firing between different neurons during the different ictal phases of seizures. To monitor synchronization of action potentials between different neurons, we calculated the cross-correlation function between the spike trains of neuronal pairs, which measured the probability of one neuron to fire as a function of the time lag from firing of the second neuron in the pair (Salinas and Sejnowski 2001).

Under control conditions, neurons tended to fire synchronously and the cross-correlograms showed significant peaks at time lag of 0 ms. On average, under control conditions, if one neuron fired, the probability of a different neuron to fire within 1 ms was 3.13 ± 0.34-fold greater than expected from chance occurrence (Fig. 5A; 1,468 neuronal pairs from 284 neurons in 26 rats). Moreover, under control conditions, significant correlations (significant positive peak in the cross-correlation function) were observed in 97.7 ± 2% of all recorded neuronal
pairs (Fig. 5B). Our experiments were performed in anesthetized rats. However, previously we have shown that widespread synchronization occurs in control awake rats as well (Cymerblit-Sabba and Schiller 2010).

To our surprise, during the initial low-amplitude rhythmic β-ictal phase the cross-correlation function showed a reduction in the tendency of neurons to fire synchronously compared with control conditions. On average, during the initial low-amplitude rhythmic β-ictal phase, if one neuron fired, the averaged probability of a different recorded neuron to fire within 1 ms was 1.81 ± 0.11-fold greater than expected from chance occurrence (Fig. 5A; 1,468 neuronal pairs from 284 neurons in 26 rats; \(P < 0.01\) compared with control). However, synchronization was not uniform in all neuronal pair. During the low-amplitude rhythmic β-ictal phase, firing of 59.4 ± 7.7% of all neuronal pairs was uncorrelated (no significant peak on the cross-correlograms) compared with only 2.3 ± 2% of uncorrelated neuronal pairs under control conditions (Fig. 5B). The remaining 40.6% of neuronal pairs did demonstrate significant correlated firing, with 8.6 ± 4.2% of neuronal pairs showing a high degree of correlated firing (exceeding a 5-fold peak on the cross-correlation function; Fig. 5C). Concomitantly, during the early β-rhythmic phase of seizures, the time window for synchronization decreased. This is shown by a significant reduction in the half-width of the cross-correlograms during the initial low-amplitude rhythmic β-ictal phase.
compared with control conditions (Fig. 5D; half-width was only measured in positively correlated neuronal pairs).

As seizures progressed and intensified, correlation of action potential firing between neurons gradually increased and spread in the cortical network. During the second low-frequency sharp-wave ictal phase, the fraction of neuronal pairs with uncorrelated firing (no significant peak on the cross-correlograms) decreased to 29.7 ± 7.8% of all neuronal pairs (708 neuronal pairs from 145 neurons in 13 rats; Fig. 5B). Concomitantly, 14.4 ± 7.6% of the stably recorded neuronal pairs showed enhanced correlated firing compared with control conditions during the second ictal phase (Fig. 5B). During the third most intense ictal phase that manifested as ECoG polyspikes, synchronization spread to involve the entire network. During this third ictal phase, if one neuron fired, then the averaged probability of a different recorded neuron to fire within 1 ms was 5.61 ± 0.39-fold larger than expected from chance occurrence (Fig. 5A; \(P < 0.01\), compared with both control and earlier ictal phases).

Similar general results were also observed during seizures induced by local intrahippocampal application of chemoconvulsants. However, in locally induced seizures, synchronization developed earlier in the course of seizures (Fig. 5). During the initial low-amplitude rhythmic \(\beta\)-ictal phase, only 12.7 ± 5.2% of all neuronal pairs showed uncorrelated firing, and synchronization spread to involve the entire cortical network during the second rhythmic spike and third polyspikes ictal phases of locally induced seizures (Fig. 5, A–C).

To further characterize the dynamics of network synchronization during seizures, we examined the changes of the cross-correlograms in individual neuronal pairs that were stably recorded during both control conditions and seizures (838

Fig. 4. Action potential firing of neurons during the different ictal phases of seizure induced by systemic pilocarpine and local intrahippocampal chemoconvulsants. In these experiments, seizures were induced either by intraperitoneal application of pilocarpine (300 mg/kg; A–D) or by local intrahippocampal application of a mixture of kainate, pilocarpine and picrotoxin (E–H). A and E: present the average (means ± SE) firing rates of neurons presented as percent of the average control firing rates during the different ictal phases. B and F: percentage of neurons that increased, decreased, or did not change their firing rates during the different ictal phases of seizures. C and G: average (means ± SE) interspike interval (ISI) during control conditions and the 3 different ictal phases. D and H: median (±SE) ISI during control conditions and the 3 different ictal phases of seizures. Data regarding systemic-induced seizures (A–D) come from 304 neurons in 26 rats. Presented data regarding local intrahippocampal-induced seizures (E–H) comes from 245 neurons in 21 rats. Note that all ictal phases were associated with an increase in the average firing frequency of action potentials. As seizures progressed, the average firing frequency further increased, and increased firing involved almost the entire neuronal population. **\(P < 0.01\).
neuronal pairs in 207 neurons from 23 rats were stably recorded during control conditions and the initial ictal phase of seizures). The results are shown for individual stably recorded neuronal pairs (Fig. 6) and for the entire neuronal population (Fig. 7). We found that 74.5% of stably recorded neuronal pairs showed a significant reduction in the peak of the cross-correlograms during the initial ictal phase compared with control conditions (at the \( P \leq 0.05 \) level) with 61.6 \( \pm \) 6.2% of these neuronal pairs showing uncorrelated firing altogether (no significant peak on the cross-correlograms). In 15.8 \( \pm \) 5.7% of neuronal pairs, the cross-correlogram peak did not significantly change compared with control conditions (at the \( P > 0.05 \) significance level), and most interestingly 9.8 \( \pm \) 5.1% of stably recorded neuronal pairs showed enhanced correlated firing compared with control conditions (peak of the cross-correlograms was significantly higher than that recorded under control conditions; Fig. 7A). These findings indicated that although widespread synchronization that characterized the normal control state was lost, synchronization of firing increased in a fraction of neurons during the early rhythmic \( \beta \)-ictal phase of seizures.

As seizures progressed, synchronization gradually increased. The number of stably recorded pairs that showed enhanced correlation compared with control conditions gradually increased, and concomitantly the number of neuronal pairs with reduced or unchanged correlation gradually decreased (Fig. 7, A–C). During the third most intense ictal phase, which manifested as ECoG polyspikes, enhanced synchronization spread to involve the entire network, and 98.2 \( \pm \) 1.4% of all stably recorded neuronal pairs demonstrated enhanced synchronization compared with control conditions (Fig. 7, B and C).
During the initial ictal phase in seizures evoked by systemic pilocarpine, rhythmic β-activity was observed intermittently (Fig. 1). We compared the average peak cross-correlograms of neuronal pairs during segments with and without the rhythmic β-ECoG activity. Synchronization was significantly increased when rhythmic β-activity was observed by the difference was not large. When the ECoG showed rhythmic β-activity, if one neuron fired the averaged probability of a different recorded neuron to fire within 1 ms was 1.95 ± 0.13-fold greater than expected from chance occurrence compared with 1.71 ± 0.09% when no accompanying rhythmic β-activity was observed on the ECoG (P < 0.05, 284 neurons in 26 rats).

We next investigated the relationship between the physical distance between neurons and their tendency to synchronize during development of systemic pilocarpine-induced seizures. We divided neurons to closely spaced neighbors that were recorded with the same electrode and more distant neurons that were recorded with different electrodes (the distance between the tips of the electrodes before insertion ranged from 200 to 500 μm). To reduce undersampling, we only included a maximal of 2 units per electrode that were sorted after separate offline runs of the raw data with different threshold values (either positive or negative). Figure 8 demonstrates that during the early low-amplitude rhythmic β-ictal phase of seizures closely neighboring neurons due tend to show a greater degree of synchronization than more distant neurons (Fig. 8). However, this difference was relatively small. In contrast, during control conditions and the more intense polyspikes ictal phase, we found no significant difference in synchronization between closely neighboring and more distant neurons (Fig. 8). These findings indicate that during the early ictal phase of seizures closely neighboring neurons have a higher probability to synchronize. However, this is not true for all closely neighboring neurons. Moreover, synchronization can also occur between more distant neurons during the early phase of seizures (the measurements were limited up to 500 μm in our experiments).

Synchronization can be affected by anesthesia, and thus we performed a set of experiments in awake rats (6 rats, see METHODS). Figure 8 presents the results of these experiments. The results obtained in awake rats were in line with those obtained in anesthetized rats. Similar to anesthetized rats, awake rats showed an increase in the average firing rates during all ictal phases (Fig. 9A; see also Cymberlit and Schiller, 2010). More importantly, during the initial rhythmic β-ictal phase, the majority of neurons showed uncorrelated firing (Fig. 9, B and C).

The probability of neurons to fire simultaneously is determined by both the firing frequency and the selective tendency of neurons to fire in synchrony. We found that the probability of neuronal pairs to fire simultaneously significantly increased during all ictal phases seizures (Fig. 10). The probability of neuronal pairs to fire simultaneously within a 1-ms time window was 0.022 ± 0.006% under control conditions. This probability increased to 0.11 ± 0.03% during the early rhythmic β-ictal phase of systemic pilocarpine-induced seizures. The probability of neuronal pairs to fire simultaneously increased already during the early ictal rhythmic β-ictal phase despite that the overall reduction in network synchronization...
was caused by the increase in the firing frequency of neurons. The probability of neuronal pairs to fire simultaneously increased to 0.19 ± 0.04% during the second periodic low-frequency sharp wave ictal phase and further increased to 0.73 ± 0.09% during the third polyspike ictal phase (Fig. 10A). Similar results were observed during local intrahippocampal chemovonvulsant-induced seizures, and the probability of neuronal pairs to fire simultaneously gradually increased as seizures progressed (Fig. 10B).

DISCUSSION

This study yielded two main findings: first, we found that epileptic seizures represented a hyperactive state manifesting as widespread increased action potential firing. Increased firing was evident in most neurons already during the early stages of seizures and further spread and increased during the later more intense ictal phases. Second, we found that during the early stages of seizures the widespread synchronization existing under control conditions was lost. We did, however, find that a minority of neuronal pairs (~10%) developed enhanced synchronization of action potential firing already during the early phase of seizures. As seizures progressed, enhanced synchronization gradually spread until it involved the entire cortical network.

The major goal of this study was to directly characterize network synchrony during seizures in vivo at the level of single-cell firing by simultaneously recording action potential firing in multiple neurons. The dogma in the field of epilepsy is that seizures result from development of a transient hypersynchronous state in the cortical network (Fisher et al. 2005; Engel and Pedley 2008). However, this conclusion is based almost exclusively on EEG and ECoG recordings. Yet, the EEG and ECoG are gross electrophysiological signals that are mostly influenced by widespread subthreshold synaptic poten-

This study yielded surprising results. In contrast to the commonly held view, we found that the average overall net-work synchronization was reduced during the early phase of seizures. Instead, we found that hypersynchrony initially developed in a small subset of neuronal pairs. This may be caused either by the fact synchronization developed initially in a subpopulation of neurons or alternatively that the network was subdivided into multiple semi-independent subnetworks with a high degree of intragroup synchronization and a low degree of intergroup synchronization. Synchronization was more likely to occur in closely neighboring neurons, suggesting small world network topology during the early phase of seizures. With time as seizures evolved hypersynchrony spread to involve the entire cortical network. Enhancement and spread of synchronization were probably responsible for the intensification of seizure activity with time.

In this study, we did not address the mechanisms underlying the tight temporal synchronization between neurons we observed under control conditions and during seizures. Moreover, we did not address the question of whether synchronization under control conditions and during seizures is mediated by the same mechanisms. Possible mechanisms that can underlie such a tight temporal synchronization are nonsynaptic mechanisms, yet such mechanisms mostly effect closely spaced neurons, while in our case more distant neurons show a similar phenomenon as well. It is important to stress that a previous study has shown long-range millisecond synchronization between cortical neurons (Roelfsema et al. 1997).

In a previous study, we found that the preictal state preceding seizures was characterized by a dramatic desynchronization of action potential firing between different neurons followed by a second phase of resynchronization (Cymerblit-Sabba and Schiller 2010). Similarly, preictal desynchronization was also reported in human seizures using EEG recordings (Mormman...
et al. 2003). The present study concentrates on the evolving network dynamics during the ictal phase. Combining our previous preictal findings (Cymerblit-Sabba and Schiller 2010) together with our new ictal findings points to the following events during development of chemoconvulsant-induced seizures. Initially, the network undergoes widespread desynchronization followed by gradual resynchronization of the network. Resynchronization initially develops in separate neuronal subgroups and gradually spreads to involve the entire network as seizures progress. The transition to the ictal state with initiation of seizures probably occurs when hypersynchronization involves a sufficient fraction of the network.

Some aspects of our findings regarding the dynamics of development of hypersynchrony during seizures are consistent with previous reports. Desynchronization of action potential firing between neurons is consistent with the report of a previous study in brain slices in vitro (Netoff and Schiff, 2002). Development of hypersynchrony in a subgroup of neurons during the early phase of seizures is consistent with the results of a recently published study in hippocampal brain slices in vitro that reported preictal synchronous firing of small groups of CA1 pyramidal neurons (Jiruska et al. 2010).

This study was performed on chemoconvulsant-induced seizures in rats. It is, however, important to stress that the ECoG of these chemoconvulsant-induced seizures shared several features with human partial seizures. Similar to chemoconvulsant-induced seizures in rats, many mesial temporal and neocortical human partial seizures initiated with a low-amplitude rhythmic $\beta$- or $\beta$-$\gamma$-activity (Salanova et al. 1992; Bartolomei et al. 2001; Tassai et al. 2002; Allen et al. 1992; Spencer et al. 1992; Alarcon et al. 1995; Schiller et al. 1998; Jung et al. 1999; Lee et al. 2000; Wendling et al. 2003; de Curtis and Gnatkovsky 2009). Low-amplitude rhythmic $\beta$- or $\beta$-$\gamma$-activity in human seizures was not distinctive of the specific pathology causing epilepsy; however, it was frequently localized to the site of seizure onset (Fisher et al. 1992; Gotman et al. 1995; Guggisberg et al. 2008). In addition similar to chemoconvulsant-induced seizures, spikes and polyspikes with or without accompanying slow-waves were commonly seen in human seizures especially during the later phases of seizures and when partial seizures secondary generalized (Fisch 1999; Kaplan and Tatum 2008; Sperling and Clancy 2008). It is important to stress that many human partial seizures are accompanied only by rhythmic activity in various frequencies and never develop ECoG spikes.

Rhythmic $\beta$-$\gamma$-activity was the first ictal ECoG manifestation in the seizures we recorded. In our experiments, we recorded from a very limited region of the CA1 hippocampal subfield. Hence, we could not determine the site of seizure onset. Previous studies reported by Bragin et al. (1999) suggest that seizures in our models initiated in extrahippocampal structures. In any case, we recorded the ECoG and unit activity in the same electrode. Thus our recordings manifest a tight correlation between the local ECoG appearance and unit activity at the same region.

In this study, we did not investigate the cellular mechanisms responsible for development of rhythmic $\beta$- and $\beta$-$\gamma$-activity during the early phase of seizures. Previous studies have reported that oscillatory $\gamma$-activity is commonly seen under physiological conditions and has been implicated in cognitive processes. Synchronized activity in the interconnected inhibitory interneuronal network and the interplay between inhibitory interneurons and excitatory principle pyramidal cells have been reported to mediate physiological $\gamma$-activity in both the hippocampus and neocortex (for review, see Whittington and Traub 2003; Bartos et al. 2007). The mechanisms underlying the low-amplitude rhythmic $\beta$- and $\beta$-$\gamma$-activity during seizures have been less extensively studied. However, two elegant recent studies have addressed this question in vitro. A recent study performed on hippocampal brain slices has shown that the very fast preictal rhythmic activity (>100 Hz) in CA1 neurons was probably mediated by synchronous firing in small groups of excitatory pyramidal neurons (Jiruska et al. 2010). In contrast, in the entorhinal cortex of isolated guinea pig brains in vitro, the rhythmic $\beta$-activity at seizure onset was mediated by intense firing of inhibitory interneurons, while firing of the principle pyramidal neurons at that time was markedly depressed (Gnatkovsky et al. 2008; de Curtis and Gantkovsky 2009).

Interestingly, in addition to the ictal low-amplitude rhythmic $\beta$-activity observed at seizure onset, the “epileptic brain” produces another type of pathological high-frequency activity. Previous studies have reported the existence of runs of high-
frequency oscillatory activity (100–200 Hz) and fast ripples (200–600 Hz) in the epileptogenic zone of both animal models and human patients during the interictal period. The mechanisms underlying these interictal high frequency discharges are yet unknown. However, they are highly localized to the epileptogenic zone and as such probably could serve to guide epilepsy surgery (for review, see Engel et al. 2009; Bragin et al. 2010).

Our findings showed that epileptic seizures are associated with a hyperactive network state. A prominent increase of action potential firing was observed in the majority of neurons already during the early ictal phases and was further enhanced and spread to involve almost all neurons as seizures progressed and intensified. These findings are consistent with those reported by Bower and Buckmaster (2008) in chronic experimental epileptic seizures in vivo. Interestingly, our previous study showed that increased firing preceded initiation of seizures (Cymerblit-Saba and Schiller 2010).

In this study, we did not investigate the cellular mechanisms responsible for hypersynchronizing the network during seizures. Several such potential mechanisms exist, especially those involving GABAergic inhibitory interneurons and regenerative dendritic mechanisms. GABAergic interneurons form a tightly linked inhibitory network interconnected by gap junctions. As a result, inhibitory postsynaptic potentials are generated synchronously in multiple excitatory neurons and in turn can contribute to synchronization of their action potential firing (for review see Mann and Paulsen 2007).

A second mechanism that can participate in hypersynchronizing the network activity during seizures is dendritic regenerativity in general and local dendritic spikes in particular. Previous studies have shown that spatio-temporally clustered synaptic inputs generate sodium, calcium, and NMDA dendritic spikes (Schiller et al. 2000; Polsky et al. 2004; Nevian et al. 2007; Larkum et al. 2009). We hypothesize that the increased firing and hypersynchronicity in neuronal subgroups occurring during the preictal and early ictal state facilitate initiation of dendritic spikes. In turn, these dendritic spikes generate correlated bursts of action potentials, and further enhance both firing and synchrony in the network. Consistent with this possibility is our previous finding that dendritic calcium spike initiated during epileptiform discharges in brain slices in vitro (Schiller 2002). In the future, we plan additional experiments to investigate the role of dendritic calcium and NMDA spikes in development and spread of hypersynchrony in the cortical network during seizures.

REFERENCES


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