Dynamics of high-frequency synchronization during seizures

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Krishnan GP, Filatov G, Bazhenov M. Dynamics of high-frequency synchronization during seizures. J Neurophysiol 109: 2423–2437, 2013. First published February 20, 2013; doi:10.1152/jn.00761.2012.—Pathological synchronization of neuronal firing is considered to be an inherent property of epileptic seizures. However, it remains unclear whether the synchrony increases for the high-frequency multiunit activity as well as for the local field potentials (LFPs). We present spatio-temporal analysis of synchronization during epileptiform activity using wide-band (up to 2,000 Hz) spectral analysis of multielectrode array recordings at up to 60 locations throughout the mouse hippocampus in vitro. Our study revealed a prominent structure of LFP profiles during epileptiform discharges, triggered by elevated extracellular potassium, with characteristic distribution of current sinks and sources with respect to anatomical structure. The cross-coherence of high-frequency activity (500–2,000 Hz) across channels was reduced during epileptiform bursts compared with baseline activity and showed the opposite trend for lower frequencies. Furthermore, the magnitude of cross-coherence during epileptiform activity was dependent on distance: electrodes closer to the epileptic foci showed increased cross-coherence and electrodes further away showed reduced cross-coherence for high-frequency activity. These experimental observations were re-created and supported in a computational model. Our study suggests that different intrinsic and synaptic processes can mediate paroxysmal synchronization at low, medium, and high frequencies.

SYNCHRONIZATION of the neuronal activity in the brain can manifest itself as both physiological and pathological processes. Physiological synchronized oscillations during sleep and wakefulness have been proposed to be involved in memory consolidation, attention, reactivation, and communication between different brain areas (Buzsáki 1989; Llinas et al. 1991; Lytton and Sejnowski 1991; Sirotta et al. 2003; Steriade and Timofeev 2003). Pathological synchronization underlies many neurological disorders such as Parkinson’s disease and epilepsy. The exact cause of the pathological synchronization is largely unknown. There are many well-known factors, such as changes in extracellular ionic concentrations or changes in the balance between excitation and inhibition, which can contribute to synchronization of the local neuronal networks. Synchronized neuronal epileptiform firing can be induced by ion concentration changes: increase in extracellular K\textsuperscript{+} concentration ([K\textsuperscript{+}]\textsubscript{o}) (Lewis and Schuette 1975; Traynelis and Dingledine 1988), decrease in [Ca\textsuperscript{2+}]\textsubscript{o} (Heinemann et al. 1977; Jefferys and Haas 1982), and decrease in [Mg\textsuperscript{2+}]\textsubscript{o} (Anderson et al. 1986). Epileptogenic drugs such as penicillin, strychnine, bicuculline, and 4-aminopyridine (Avioli et al. 1993; Hotson and Prince 1981; Lerma et al. 1984; Matsumoto and Marsan 1964; Prince 1968a, 1968b; Wong and Prince 1979) act through modulation of synaptic transmission or intracellular properties to produce epileptiform activity. Electrical stimulation or kindling is another common approach to trigger epileptiform discharges (Bragin et al. 1997; Stasheff et al. 1985).

Once synchronization of the neuronal firing reaches a certain threshold, it manifests itself as seizures, which is a keystone of epilepsy. While epileptogenic factors that promote epileptiform activity are somewhat known, the exact mechanisms that result in pathological synchronization during seizure are still under investigation.

Spectral frequency analysis using fast Fourier transform (FFT) and wavelet methods is a common approach to gain insight into the mechanisms of synchronization (Akin 2002). Oscillations at alpha, beta, gamma, delta, and theta frequency bands of neuronal activity (Canan et al. 2008; Dugladze et al. 2007; Timofeev and Bazhenov 2005; Timofeeva and Gordon 2001) have been thoroughly investigated, but mechanisms of synchronization at higher frequency bands remain elusive. Ripples and fast ripples are well-studied high-frequency oscillations in mice, rats, primates, and humans, mainly in hippocampal CA1 and CA3 regions (Bragin et al. 1999a; Buzsáki et al. 1992; Chrobak and Buzsáki 1996; Csicsvari et al. 1999; Ylinen et al. 1995). Ripples precede epileptiform activity (Bragin et al. 1999b; Grenier et al. 2001) and are usually localized in brain regions involved with spontaneous seizures (Worrell and Gotman 2011). Propagating high-frequency oscillations have been reported in the human and rat neocortex (Kandel et al. 1997; Staba et al. 2003, 2004). However, previous studies on ripples have limited the analysis to frequencies of 500 Hz or less (Bragin et al. 1999a), referring to activity above this frequency as noise.

In this study we analyzed hippocampal spatio-temporal dynamics, using wide-band spectral analysis (up to 2,000 Hz) of neuronal activity during epileptogenesis. We utilized novel perforated multielectrode array (MEA) recordings, which allowed us to resolve single-unit activity and local field potentials (LFPs) at up to 60 locations throughout the mouse hippocampus. During epileptogenesis we found spatio-temporally distributed patterns, represented by characteristic LFP profiles: decreased cross-coherence between different locations of the hippocampus during bursting at the high frequency bands (500–2,000 Hz), unchanged at middle frequencies (100–500 Hz), and increased at low frequencies (0.1–100 Hz). We speculate about possible mechanisms underlying this decrease in cross-coherence at high frequencies. Our study provides new insights into spatio-temporal dynamics of pathological hippocampal network synchronization and reveals essential properties of high-frequency synchronization in mouse hippocampus during epileptiforms.
METHODS

Preparation of hippocampal slices. Acute hippocampal slices were prepared from FVB-Tg(Gad GFP)45704SwmJ postnatal day (P)14–40 mice (JAX Labs). Mice were anesthetized and decapitated according to the University of California, Riverside IACUC-approved protocols. The brain was rapidly removed and sliced (300 μm) with a Leica200 vibratome in ice-cold low-[Ca\(^{2+}\)]\(_{\text{aq}}\), high-sucrose dissection solution. The slices were recovered in normal artificial cerebrospinal fluid (ACSF) for 1 h at 32°C and finally stored at room temperature.

Solutions. During recordings, brain slices were continuously perfused in ACSF (5 ml/min flow rate) containing (mM) 125 NaCl, 2.5 KCl, 25 NaHCO3, 1.25 NaH2PO4, 1 MgCl2, 2 CaCl2, 25 α-glycerophosphate, and 10 sucrose, pH 7.4. We maintained continuous oxygenation with a 95% O2-5% CO2 gas mixture (osmolarity 290 mOsm/l). The standard dissecting solution contained (mM) 87 NaCl, 2.5 KCl, 1.25 NaHCO3, 0.5 MgCl2, 0.5 CaCl2, 10 sucrose, and pH 7.4, and was maintained by continuous oxygenation with a 95% O2-5% CO2 gas mixture. All chemicals were from Fisher Scientific unless otherwise specified. Continuous epileptiform activity was triggered by raising [K\(^{+}\)]\(_{\text{aq}}\), from 2.5 mM (normal ACSF) to 10 mM. This approach leads to characteristic electrical activity that is typical for electrographic seizures, and therefore we believe our findings are applicable to other epileptiform initiation methods.

Multielectrode array recordings. MEA recordings were performed with a 60-channel perforated array (Filatov et al. 2011) and a low-noise amplifier (Multi Channel Systems). Perforated arrays allow the electrodes to reach undamaged areas. The data presented in this study were obtained from a MEA with 200-μm interelectrode distance. Hippocampal slices were prepared as described above and placed on the array to cover the area of interest. All MEA recordings were done at 32.5 ± 0.5°C, which was enough to induce stable epileptiform. Higher temperatures lead to more severe epileptiform and faster slice degradation. Data were acquired and preliminarily analyzed with MC Rack software (Multi Channel Systems) and exported to pCLAMP 10 (Molecular Devices) and MATLAB (MathWorks) for final analysis.

Signal processing of MEA data, event detection. Data from all 60 channels of the MEA were recorded at 25,000 Hz and then downsampled to 5,000 Hz for further analysis. Channels with artifacts or high noise level for the entire duration of the recordings were removed after visual inspection. To detect synchronized network events (referred as “burst events” or “bursts” in the text below) in MEA recordings, first the channel with the highest amplitude was detected with a percentile-based method applied on the unfiltered data. In this method, a high (98.75 ± 0.75, varied across slices) and a low (1.25 ± 0.75) percentile were measured for each channel and then the channel that had the maximum absolute value between high and low percentile values was chosen. The high percentile value corresponds to the positive polarity response, while the low percentile value corresponds to the negative polarity response. The percentile method prevents erroneously picking a channel that had high-amplitude activity at only a few time points. The amplitude corresponding to the high or low percentile was used as the threshold for detecting burst events. The percentile method offers a number of advantages over the standard RMS method. The percentile method is not influenced by the artifacts that would occur because of movement or electrical spikes. This is a nonparametric method, and therefore only the order of amplitudes plays a role and not their absolute value. Nonparametric methods have been shown to be better suited for statistical analysis, especially when the distribution could be influenced by a few tail values located far from the distribution median. The accuracy of this method is illustrated in Fig. 1C.

The onset of a burst event was detected as a time moment when the amplitude of the LFP exceeded threshold defined with the percentile method as described above. Burst events were then extracted from all channels as periods of 500 ms prior to and after the time point when epileptiform activity crossed threshold. To prevent overlapping of burst events, only events separated by >500 ms were considered, thus setting a maximum possible oscillation frequency of 2 Hz. The performance of this algorithm was further confirmed by visual inspection of the detected events, and it worked well across all slices. The channel that had the maximum high or low percentile threshold value was chosen as the seed channel for delay and cross-coherence analyses. Baseline subtraction (300–400 ms prior to the threshold point was used as a baseline period) and linear detrending in MATLAB (MathWorks) were performed on each burst event for all channels. Current source density (CSD) on a two-dimensional grid was computed as the second discrete partial derivative, similar to methods used in the past (Mitzdorf and Singer 1978).

Delay analysis. Delays between the seed channel and all other channels were computed for five broad frequency bands: 0.1–10 Hz, 10–100 Hz, 100–500 Hz, 500–1,000 Hz, and 1,000–2,000 Hz. We first computed power and phase coherence across the entire spectrum (with 10-Hz resolution for 0.1–2,000 Hz) for all slices and all channels, and then we selected five frequency bands based on the results for the main analysis.

Delay between the seed channel and every other channel was computed based on the cross-correlation of power obtained with Hilbert transform. Hilbert transform was applied on narrow-band filtered activity. We used a square linear-phase FIR filter (MATLAB) with a fixed order of 300 for the narrow-band filters. First, we computed a cross-correlation as a function of lag time from the time series of power of the seed channel and all other channels for each burst. Then the time lag corresponding to the maximum of the cross-correlation function was taken as the delay for each burst. Finally, the average delay across all bursts was used for further plots and statistical analyses. The measured delay was specific to the particular frequency band. This method for detecting delays between LFPs has been compared with other techniques and showed better performance when dealing with noisy data (Adhikari et al. 2010).

Cross-coherence. Phase-based cross-coherence was used in this study. Intuitively, this measure computes the consistency of the phase difference between channels for a given frequency band and time point. The consistency is measured by averaging the phase difference between channels across trials for the same frequency and time. The resultant phase cross-coherence measure has a range from 0 to 1, where 0 means no consistency (or highly variable phase differences across trials) and 1 means the same phase difference between channels measured across all trials for the given frequency and time. In strict terms, the phase cross-coherence was computed between the seed channel and every other channel as $1/K \left[ \sum_{i=0}^{K} \left. \frac{\text{ht}_i}{\text{ht}_i} \right| \frac{\text{ht}_i}{\text{ht}_i} \right]$, where $K$ is the number of burst events, $\text{ht}$ is the Hilbert transformation for a given channel, $\text{ht}_i$ is the Hilbert transformation of the seed channel, and $\text{hts}$ is the complex conjugate of $\text{ht}_i$. In other words, the phase cross-coherence measures the average of phasors, where phasors are unit amplitude complex vectors obtained from the product of complex-valued Hilbert transformations of two channels for a given frequency and time. Similar measures have been used by Lachaux et al. (1999). The Hilbert transformation was computed after using a narrow-pass band FIR filter for frequencies from 0.1 to 2,000 Hz with 10-Hz steps for the entire duration of each burst event (1,000 ms). The filter parameters were the same as described in the previous section. Linear detrending was performed for each trial and channel prior to performance of the cross-coherence analysis to remove the effect of difference in amplitude between channels as the source of bias in cross-coherence analysis.

The cross-coherence values for five frequency bands (0.1–10 Hz, 10–100 Hz, 100–500 Hz, 500–1,000 Hz, and 1,000–2,000 Hz) were computed by averaging from the cross-coherence computed with 10-Hz steps. For further analysis, cross-coherence values were also averaged in time to compare time periods before and during the event. Time points between −400 and −240 ms (0 ms is the time of threshold crossing) were used to compute cross-coherence before the event.
event, and the −60 to 100 ms time period was used to compute cross-coherence during the event. Since 0 ms corresponds to the time moment of crossing the threshold, the event’s onset was slightly earlier, justifying the use of the −60 to 100 ms time period for measuring cross-coherence during the event. Selected time periods prior to and during the burst event had the same duration. These time periods were chosen on the basis of visual inspection of the timing of the burst events across all channels and slices. The difference in cross-coherence prior to the event and during the event was used as the change in cross-coherence for each channel.

While we observed a 60-Hz noise artifact with its harmonics at 240 Hz, 300 Hz, etc., in some slice/channel combinations, reduction in high-frequency coherence reported in this study was broadband and not restricted to the 60-Hz fundamental or its harmonics. Furthermore, the slices and channels without or with only a very low 60-Hz artifact also showed qualitatively similar cross-coherence changes at high frequencies.

Statistical analysis. A paired t-test was used to compare cross-coherence calculated before the burst and during the burst. Correlation with Pearson’s correlation coefficient and its significance was used to identify the relationship between delays and distances between channels. All statistical tests were done in MATLAB (MathWorks).

Network model of \([K^+]_o\). Pyramidal cells (PYs) and fast-spiking inhibitory interneurons (INs) were modeled as two-compartment neurons with axo-somatic and dendritic compartments described with a conductance-based approach. This model is derived from previous models of epileptiform activity with high extracellular potassium (Bazhenov et al. 2004; Fröhlich et al. 2006; Krishnan and Bazhenov 2011; Mainen and Sejnowski 1996). The main change from previous models was including the effect of maintaining persistent high \([K^+]_o\), to mimic the presence of the unlimited supply of \([K^+]_o\) in the bath solution in vitro. This was implemented by modifying the \([K^+]_o\) dynamic equations by the following equations:

\[
d\frac{[K^+]_o}{dt} = \frac{k}{F_d} \left( \frac{[K^+]_o}{[K^+]_{pump}} + G \right) + G + \delta_g \left( \frac{[K^+]_{c-1} + [K^+]_{c+1} - [K^+]_c}{2} \right) + \delta_l [K^+]_{oc} - [K^+]_o + \frac{K^+_{bath} - [K^+]_o}{\tau_{bath}}
\]

\[
G = \frac{k_1 (B_{max} - [B])}{K_{1N}} - k_2 [K^+]_o [K^+]_o
\]

\[
d\frac{[B]}{dt} = k_1 ([B]_{max} - [B]) - k_2 [K^+]_o [B]
\]

where \([K^+]_{bath}\) is the concentration of \([K^+]_o\) maintained in the bath solution, \(\tau_{bath}\) is the time constant of the \([K^+]_o\) concentration change in the bath, \(k (= 10)\) is a conversion of \([K^+]_o\), and \(k_1 = 0.008\), respectively. \(k_2 = k_1 / \left(1 + e^{(K^+_{c-1} - K^+_{c-1(\text{min})}) / 1.15}\right)\). \(F = 96,489 \text{C/mol, } d = 0.15\) determined the ratio of the volume of the extracellular compartment to the surface area, \(I_C\) is the concentration of intrinsic current to \([K^+]_o\), \(I_{pump}\) is the pump contribution to \([K^+]_o\), \(I_{oc}\) is the concentration of \([K^+]_o\) in the adjacent compartment, and \([K^+]_{c-1} \text{ and } [K^+]_{c-1} \text{ are the respective concentrations of potassium for the neighboring cells. } G\) corresponds to the glial contribution with its buffer (B) and maximal buffer of \([B]_{max} \text{, Intracellular } [Na^+] \text{, } [Cl^-], \text{ and } [K^+] \text{ and extracellular } [Na^+] \text{ were the dynamic variables in the model as described by Krishnan and Bazhenov (2011). Other details of the model are similar to the previously published model (Fröhlich et al. 2006).}

We simulated 1) small (10 PY neurons and 1 IN) and globally connected networks and 2) large (100 PY neurons and 20 INs) and locally connected networks. In all models, an external source of \(K^+\) maintained \([K^+]_o\) at specific levels to mimic the conditions of an in vitro experiment. The small network had global connectivity between PYs with AMPA and NMDA connections. The only IN was connected to all the PYs with GABA_\text{A} synapses, and all PYs had AMPA projections to the IN. In a larger network of 100 PYs and 20 INs, each PY had projections to 10 neighboring PYs and 1 IN. Each IN received input from 5 PYs and projected back to 5 PYs. Variability was introduced to the network by slightly varying the leak conductance of chloride leak currents across PYs (from a Gaussian distribution with a mean of 0.44 mS/cm^2 and SD of 0.044 mS/cm^2). Other forms of variability (maximal conductance of potassium leak current or independent external Poisson noise) were also tested in the model and produced similar results.

RESULTS

Statio-temporal patterns of epileptiform activity. In this study, persistent epileptiform activity was triggered by raising bath potassium concentration ([K^+]_o) from 2.5 mM (normal ACSF) to 10 mM. Increase of [K^+]_o led to the rise of multunit activity followed, after a 2- to 5-min delay, by the appearance of stable and slow (<1 Hz) network bursting (Fig. 1A). All bursting events recorded within the same channel showed stereotyped temporal structure over the entire seizure duration (Fig. 1, B–D). Burst duration varied between 100 and 300 ms (Fig. 1, D and E). The frequency of bursting was ~0.2–2 Hz (Fig. 1B) and varied based on [K^+]_o. Once [K^+]_o reached 15 mM or higher, the network oscillations came to a relatively fast halt, presumably by depolarization block. We observed negative, positive, and mixed polarity of the LFP fluctuations (Fig. 1D). The averaged spectrogram of all bursts showed increase in power during the burst events (100–300 ms) across a wide range of frequencies (Fig. 1F). The choice of the frequency bands used in our study was based on previous experimental studies, which showed distinct activity corresponding to different frequencies (Bragina et al. 1999b; Li et al. 2005; Timofeeva and Gordon 2001). In our analyses, we used 0.1–10 Hz as a low frequency band, which focused on capturing slow bursting activity. The second frequency band was 10–100 Hz, which corresponded to activity in alpha, beta, and gamma ranges and was used in several studies (for review see Buzsáki and Silva 2012). The third frequency band was selected as 100–500 Hz, which included ripple and fast ripple frequencies and could capture intrinsic multunit neuronal activity. The last two bands were 500–1,000 and 1,000–2,000 Hz. These bands have not been analyzed previously but showed robust activity during paroxysmal oscillations in our study (Fig. 1F). Indeed, the spectrogram revealed increase of activity up to 2,000 Hz during the epileptic events compared with baseline (Fig. 1F and Fig. 2D). Activity over 500 Hz is considered to arise from multunit activity and was observed across multiple channels.

MEA recordings allowed us to characterize spatio-temporal distribution of the epileptiform activity across the entire hippocampal slice (Fig. 2). We observed complex positive, negative, and bipolar LFP events in the low frequency band (0.1–10 Hz), which reflected the distribution of current sinks and sources across different hippocampal layers (Fig. 1, C and D, and Fig. 2, B and C). Because of their stereotyped spatial orientation, CA3 pyramidal neurons were major contributors to this pattern. For unfiltered signal [mainly representing low frequencies (~10 Hz)], the active phase of the network burst

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Fig. 1. Epileptiform activity in hippocampal slice induced by high extracellular $\text{K}^+$ concentration ([K$^+$]$_o$). A: continuous epileptiform activity in 60 multielectrode array (MEA) channels. B: activities of the 3 selected channels differ in polarity of the burst events. C: activity of all burst events for 1 channel superimposed on the same plot. Note that all events cross the threshold value at the same time point. D: 1 representative epileptiform event from the 3 channels shown in B. E: all burst events from the same channel (channels 45, 47, 49 are shown) aligned by the time when activity crossed threshold for the seed channel (in this case, the seed channel was channel 49). F: averaged across all burst events power spectrum for channels 45, 47, and 49. Note strong signal up to 2 kHz.
(100–200 ms in Fig. 2C) consisted of a negative LFP in the stratum pyramidale of CA3 and a positive LFP in the areas of apical and proximal CA3 dendrites (Fig. 2C, top). At the end of the burst event (200–300 ms in Fig. 2C), LFP polarity was reversed, so the stratum pyramidale of CA3 showed a positive LFP while the stratum radiatum showed a negative LFP polarity. At high frequencies (100–500 Hz and 500–1,000 Hz), only the channels around stratum pyramidale of CA3 region were active (Fig. 2D). Furthermore, high-frequency activity (100–1,000 Hz) appeared only in the narrow time window during the burst event (100–250 ms in Fig. 2D). Finally, at very high frequencies (1,000–2,000 Hz), the activity was
widespread across the entire hippocampus slice and recording time (Fig. 2D, bottom).

CSD analysis was used to identify the current sources for various frequency bands. To avoid averaging at high frequencies, we present snapshots of the CSD distribution at selected time moments rather than an average over time. CSD analysis revealed that at low frequencies (0.1–10 Hz) the stratum pyramidale of CA3 was the prominent current sink while distal dendrites were the source (Fig. 3B). CSD was reversed at ~200 ms, corresponding to the time moment of the burst termination (Fig. 3A).

We then used low-frequency CSD (0.1–10 Hz) over the 100–200 ms time period to cluster electrodes into the groups of similar activity (Fig. 2A, right). In groups i and ii the electrodes were sinks at the beginning of the burst and sources later, while group iv showed the opposite pattern (Fig. 3A). These cluster groups revealed close correspondence to the

Fig. 3. CSD distribution in different frequency bands. A: CSD for all channels and 5 frequency bands across the entire duration of a single burst. Black lines separate 4 different groups of channels identified based on cluster analysis (Fig. 2A, right). Arrows indicate time points corresponding to snapshots of activity in B. B: CSD snapshots at selected time moments in different frequency bands.
anatomy of the CA3 pyramidal neurons. Indeed, cluster groups i and ii have been localized to stratum pyramidale of CA3, while cluster group iv was localized in the area of apical and proximal CA3 dendrites.

CSD at higher frequencies showed varied profiles. Similar to the low frequencies, in the 10–500 Hz range the maximal amplitude of currents was mainly recorded by electrodes located near CA3 pyramidal neurons. However, we observed several localized clusters of neurons firing in opposite phase (Fig. 3B). Distribution of sinks and sources changed many times over the duration of the burst (Fig. 3). At frequencies above 500 Hz, CSD was spatially diffuse without any particular spatial localization (Fig. 3B).

Analysis of delays. Delays at a given frequency between the selected seed channel and all other channels were obtained with a cross-correlation method (see Methods). The delay measured with this technique represents the average (measured across multiple bursts) time lag between the peaks of activity at a given frequency in a seed channel and another channel. The delays were computed for five different frequency bands (0.1–10 Hz, 10–100 Hz, 100–500 Hz, 500–1,000 Hz, and 1,000–2,000 Hz). While the low-frequency activity (0.1–10 Hz) evolved slowly (as shown in multiple frames in Fig. 2C, top), the high-frequency activity (100–1,000 Hz) appeared only for a brief period (Fig. 2D, top and middle). The low-frequency activity showed longer delays between electrodes (up to 70 ms) compared with the high-frequency activity (delays up to 30 ms). The delays reduced as frequency increased (Fig. 4A). Pearson’s correlation was used to measure dependence of the absolute value of delay on the distance between electrodes (Fig. 4B). In general, there was a weak correlation between distances and delays at all frequencies; however, the likelihood of finding a large delay was slightly higher for longer distance between electrodes.

The spatial plot of delays for one slice (Fig. 4C) illustrates the origin of a weak relationship between distance and delay. While in general channels that are closer to each other showed shorter delays than channels further away, some channels that displayed less activity and were located further away also showed shorter delays. This could arise because of the localized nature of epileptic foci, as seen in Fig. 2D, where only a section of the MEA grid showed large activity while several other channels remained dormant. At high frequency, because of the low amplitude of signal in channels located further away from the seed channel, delays were not consistent and the mean delay was close to 0 ms. However, even for high-frequency activity, the channels located near the seed channel displayed an inverse relationship between distance and delay (Fig. 4C). Overall, this analysis suggests that 1) high-frequency delays are smaller than low-frequency delays and 2) delays depend on distance primarily for low-frequency activity. Thus the power-phase relationship between low- and high-frequency activities depends on spatial location within the epileptiform bursts.

Frequency and distance dependence of synchronization. The degree of synchrony between different areas of the hippocampus was measured with cross-coherence analysis (see Methods). We found that cross-coherence between channels showed different temporal patterns for different frequency bands. At the low frequency bands (0.1–10 Hz, 10–100 Hz) the cross-coherence increased during the bursts, while at the high frequency bands (500–1,000 Hz, 1,000–2,000 Hz) the cross-coherence reduced during the burst events (Fig. 5A). The spatial distribution of the change in cross-coherence (difference between cross-coherence during the burst and before the burst) showed that at the low frequency (0.1–10 Hz) all channels became highly synchronized during the burst event (Fig. 5B, left). In contrast, at 100–500 Hz, 500–1,000 Hz, and 1,000–2,000 Hz frequencies only a few channels located near the seed channel showed increase in coherence while a majority of the distant channels showed reduction of coherence (Fig. 5B, center and right).

The low-frequency activity (up to 500 Hz) had a widespread cross-coherence during the bursts (Figs. 5, B and C). Cross-coherence slowly decreased with distance from the seed channel, as revealed by a negative correlation between distance and cross-coherence during events at frequencies up to 500 Hz (0.1–10 Hz: \( r = -0.21, P < 0.001 \); 10–100 Hz: \( r = -0.504, P < 0.001 \); 100–500 Hz: \( r = -0.38, P < 0.001 \)). Above 500 Hz, most channels showed reduction in cross-coherence (as shown in Fig. 5), so there was no significant correlation between cross-coherence and distance (500–1,000 Hz: \( r = -0.028, P = 0.44; 1,000–2,000 \) Hz: \( r = 0.07, P = 0.05 \)). Overall, we conclude that the low-frequency activity (0.1–10 Hz) was synchronized across all channels, suggesting underlying synchronized postsynaptic activity. The high-frequency activity, which reflects multunit activity, was synchronized only for small regions around the maximum activity channel.

Paired t-test was used to compare the average cross-coherence values prior to and during the burst (see Methods) across all channels in all experiments. There was a significant increase of coherence in 0.1–10 Hz \( [1,779] = 34.5274, P < 0.001 \) and 10–100 Hz \( [1,779] = 25.2518, P < 0.001 \) frequency bands, while there was a reduction at 500–1,000 Hz \( [1,779] = 16.8326, P < 0.001 \) and 1,000–2,000 Hz \( [1,779] = 22.1432, P < 0.001 \) frequency bands (Fig. 6). The average cross-coherence did not significantly differ at the 100–500 Hz frequency band \( [1,779] = 1.473, P = 0.141 \).

To rule out a possible bias in our choice of the seed channel, the cross-coherence was also computed for each frequency band between all possible pairs of channels. There were 4,096 (64 x 64) cross-coherence values for each frequency band, which is plotted in matrix form in Fig. 5C. This analysis confirmed that cross-coherence increased at the low frequency band (0.1–10 Hz), while at higher frequencies, with the exception of one or two clusters, all channel combinations showed reduction in coherence. This analysis also detected clustering of channels based on synchrony at each frequency band. Each such cluster indicated a group of channels displaying high average correlation during events. Surprisingly, the clusters observed for different frequency bands differed significantly in their spatial location within a slice. To independently confirm the results of correlation analysis, we performed a wavelet analysis of a subset of data (not shown). In agreement with our results reported here, wavelet analysis (Morlet wavelet with center frequency of 1 and bandwidth 1 and 12 points) revealed decrease in wavelet cross-coherence at the high frequency band (>500 Hz for remote channels) and increase at the low frequency band (<200 Hz).

Network model. To identify mechanisms involved in frequency-dependent change in cross coherence, we developed
Fig. 4. Analysis of time delays at the burst onset. 

A: plot of time delays between seed channel and all other channels at the burst onset. Five different frequency bands are shown. Each line represents the average delay across 60 channels, first sorted by delay value, then averaged across slices ($n = 12$ slices). Error bar indicates SE of the variability across slices.

B: scatterplot of delays as a function of the distance for different frequency bands. Solid line shows linear regression. Correlation coefficient and $P$ value are shown at top.

C: delays between electrodes plotted on top of the MEA grid for 5 frequency bands. In this plot, the sign indicates the order of activity and the absolute value indicates the time difference between activities in a given channel and the seed channel. Blue color (negative delay) indicates that activity of a channel has its peak before the seed channel, and red color (positive delay) indicates activity peak occurring after the seed channel. Asterisk indicates the seed channel.
simple computational models of the hippocampal network. In all models, extracellular and intracellular ion concentrations were dynamic variables, whose changes were determined by differential equations based on current flow and ionic pumps (see METHODS). In addition, an external source of K\(^+\) maintained \([K^+]_o\) at specific levels to mimic the conditions of in vitro experiments.

In the small network of 10 PYs and 1 IN, \([K^+]_o\) progressively increased during the initial period until it stabilized around 8 mM (Fig. 7). The \([K^+]_o\) increase led to the higher spiking activity of all neurons in the network. During the initial period (2–5 s after initiation of activity), PYs exhibited tonic high-frequency activity (Fig. 7D, left) followed by a long period of continuous bursting activity (Fig. 7D, right). Here tonic activity is defined as a period of continuous spiking with no depolarization block or hyperpolarization periods, while bursting activity is defined as high-frequency firing with a period of depolarization block followed by a long hyperpolarization (lasting for 100–500 ms). This pattern is typical for paroxysmal depolarization shift observed during cortical epileptic seizures (Timofeev et al. 2002; Timofeev and Steriade 2004). INs showed high-frequency spiking with no bursting (not shown). The onset of bursts appeared highly synchronous across all PYs, as seen in the space plot in Fig. 7A. The bursting activity was stable and continuous, similar to the experimental data. To investigate the degree of synchronization at low and high frequencies in the model, we selected as seed channel a neuron in the middle of the network and compared timing of bursts between different neurons and at different frequencies.

Fig. 5. Analysis of synchronization. A: cross-coherence across 60 channels at different frequency bands for a single representative slice, plotted for duration of the burst. x-axis in each plot is time in milliseconds, and y-axis is channel number from 1 to 60. Each row in the plot is the averaged across burst events cross-coherence between the seed channel and a specific channel. Each column corresponds to a particular frequency band. B: spatial profile of the cross-coherence during the burst (measured as averaged cross-coherence during 200 ms) plotted on top of the MEA grid for the same slice as in A. Asterisk marks the seed channel of maximal activity. C: cross-coherence computed between all possible channel combinations. Each row corresponds to the cross-coherence measured between that channel and all other channels. The diagonal corresponds to the cross-coherence of a channel to itself; hence it was zero. It is a symmetrical matrix since the cross-coherence is identical when channels are commuted.
In a small network with identical neurons, activities of all neurons were highly synchronized within each burst (Fig. 7E) even though the burst duration varied over the period of simulation (Fig. 7F). When variability was introduced in the network (by varying the conductance of chloride leak currents between neurons), synchronization of spiking activity (or high-frequency activity) was reduced (Fig. 7G, compare with Fig. 7E). The loss of synchrony at high frequency (spike timing) was also visible in the plot showing all burst events for three neurons (Fig. 7H, compare with Fig. 7F). In contrast to the high-frequency activity, the low-frequency activity (the envelope of the burst) remained highly synchronous across neurons (Fig. 7, G and H). The AMPA and NMDA connections between PYs were crucial for synchronization of low-frequency activity in the model. When these connections between PYs were removed, the network showed loss of synchronization in both high and low frequency bands (Fig. 7, I and J).

We then simulated a larger network with intrinsic parameter variability (Fig. 8). Similar to the small network, the neurons showed bursting activity when $[K^+]_o$ increased (Fig. 8, A–C). In agreement with experimental data, the frequency of bursting activity in the model was dependent on $[K^+]_o$. To simulate LFP, an averaged activity (hereafter referred to as an averaged potential) of every five adjacent neurons in the network was computed (Fig. 8, B, bottom, and D). Figure 8E shows averaged potential across all burst events for five representative channels. The averaged potential corresponding to the middle of the network (channel 10) was chosen as a seed channel. The degree of synchronization appeared to reduce with distance between channels (Fig. 8E). This observation was further verified by cross-coherence analysis using the same method as for experimental data. During the burst event, the cross-coherence of the averaged potentials showed reduction at high frequencies, while there was a slight increase at low frequencies (Fig. 8F). There was also a short period during the middle of the burst event when the cross-coherence remained high at all frequencies. This period corresponded to the depolarization block in the model neurons; there was no spiking activity. Cross-coherence during the burst events reduced with distance between channels: slow for low frequencies and fast for high frequencies (Fig. 8G).

To identify a possible role of fast synaptic inhibition in synchronizing network activity, we systematically varied the strength of GABA_A inhibitory synaptic synapses between INs and PYs. We found that GABA_A inhibitory synaptic connection strength influenced the level of cross-coherence at high frequencies (Fig. 8H). For low and high levels of inhibition there was a reduction in cross-coherence compared with the middle-level inhibition in the network. However, it should be noted that even for the middle level of GABA_A inhibition there was still relative reduction in cross-coherence during burst events compared with baseline activity.

DISCUSSION

We used a MEA with a spatially dense 60 channels to characterize network activity in a broad spectral range of up to 2 kHz during epileptic bursts triggered by elevated $[K^+]_o$ in mouse hippocampal slices. Our study revealed that 1) there is a prominent structure of LFP profiles with characteristic distribution of current sinks and sources predominantly in the CA3 region of the hippocampus; 2) onsets of epileptiform events across different spatial sites were characterized by much longer delays for low-frequency activity than for high-frequency activity; and 3) during epileptiform burst events cross-coherence between electrodes increased (relative to the baseline level) for low frequencies but decreased at high frequencies (attributed to multiunit activity).

LFP profiles of epileptiform activity are different across frequency bands. The overall spatio-temporal characteristics of the epileptiform activity were determined by the underlying structure of the tissue. In most slices, in the low frequency band we observed negative LFP and a current sink in the stratum pyramidale of CA3 during the burst event followed by positive LFP and a current source during silent periods between bursts. The current sink likely reflected inward depolarizing currents associated with a depolarizing envelope of the burst event and multiunit activity. The current source during hyperpolarized periods was mediated by slow hyperpolarizing currents responsible for burst termination and interevent silence. Electrodes in the stratum radiatum of CA3 showed opposite LFP polarity, with initial positivity during the burst event followed by negativity during the silent phases. The positive LFP likely reflected passive current sources (current outflow from distal dendrites) followed by current sinks in the dendrites after burst termination. The precise structure of current sinks and sources was further confirmed by two-dimensional CSD analysis. Similar findings have also been observed in the neocortex in vivo (Chauvette et al. 2010; Timofeev et al. 2000), where large positive and negative responses aligned with axonal and dendritic structures of layer V pyramidal neurons during slow cortical oscillations. In the frequency band corresponding to ripples and high-gamma activity (100–500 Hz), we observed alternations of source-sink distribution that occurred around the stratum pyramidale of CA3, suggesting the same underlying mechanisms of activity as during hippocampal sharp waves (Sullivan et al. 2011). Frequency-specific spatiotemporal analysis revealed that low-frequency activity (0.1–10 Hz) was spatially and temporally spread out compared with high-frequency activity (100–1,000 Hz). The slow activity was fo-
Fig. 7. Dynamics of epileptiform-like activity in the network model. A: activity of all neurons in the network model over the entire time of simulation. B: activity of a single representative pyramidal neuron from the network (indicated by arrow in A). C: dynamics of $[K^+]_{o}$. D: single pyramidal neuron shows tonic spiking (left) and bursting (right) behavior at different times during simulation. E: activity of all neurons during 3 different events (from top to bottom) shown as a space plot for the network with identical neurons. x-Axis indicates time, and y-axis shows index of a neuron in the network. Burst events are selected when activity crossed a threshold value of $-20 \text{ mV}$ in seed neuron 5. F: activity of 3 neurons (2, 5, and 8) from the network in E, plotted for all burst events ($n = 150$). x-Axis indicates time, and y-axis shows burst number. G and H: same plots as in E and F but for the network with nonidentical pyramidal neurons (chloride leak current was varied across neurons). I and J: same plots as in E and F but for the network of identical neurons without excitatory connections between pyramidal neurons.
cused around both dendritic and somatic regions, while higher frequencies had a peak around the stratum pyramidale of CA3 and only observed during the burst event. These differences in spatial pattern between frequencies suggest activity in the 0.1–100 Hz bands may arise from synchronized synaptic activities of the dendrites, while higher frequencies arise from fast currents involved in action potential generation in somas of CA3 pyramidal neurons.
Network synchronization during epileptiform is different in low and high frequency bands. In this study, synchrony during epileptic bursts between channels and across frequencies was measured with phase cross-coherence representing reliability (or consistency) of phase difference between two channels across many bursts. Phase cross-coherence was found to decrease at high frequencies (500–2,000 Hz) and to increase at lower frequencies (<10 Hz) during the epileptic bursts. Our study therefore suggests that multinit activity becomes desynchronized during epileptic bursts, while slow depolarizing events display a higher degree of synchrony. It should be noted that the low-frequency activity by its nature evolved more slowly compared with the high-frequency activity, which makes the synchrony measures less sensitive to small variability at low frequency compared with the high frequencies.

Analysis of delays during the burst event revealed that high frequencies occurred in a relatively narrow time window compared with low frequencies, suggesting that high-frequency activity across channels occurs relative to some shared process across the entire network while the low-frequency activity is determined by the local processes. These local processes mediate relatively slow propagation of the low-frequency activity across the network. Further studies are required to identify the mechanisms of shared processes resulting in the global (but nonsynchronized, see below) high-frequency activity.

Previous studies looking at low frequencies have shown increased synchronization during epileptic bursts (Takeishi and Bahar 2011). Research on the synchronization of spiking activity shows reduced synchrony in both direct recordings from humans (Truccolo et al. 2011) and in vitro recordings from rats (Netoff and Schiff 2002). EEG recordings also suggested reduced synchrony (Wendling et al. 2003) and increased complexity of neuronal activities during epileptic discharges (Schindler et al. 2007). Combining the results from the present and previous studies, synchronization during epileptic bursts displays complex dependence on frequency and distance from the foci. Low-frequency activity is synchronized across larger areas, while high-frequency activity is synchronized only within small regions and becomes decorrelated at long distances. Local regions with strong high-frequency synchrony may be a focal point of seizures and might be used for better detection and prediction of seizures. Further studies are needed to examine differences in progression of neuronal activity prior to seizures at these high-frequency synchronized nodes.

To identify the mechanisms behind changes in synchronization during seizures, we used a computational network model of the hippocampus. This model was based on our previous studies and included detailed ionic dynamics in the intra- and extracellular space (Bazhenov et al. 2008; Fröhlich et al. 2010; Krishnan and Bazhenov 2011). We showed that elevated [K+]o leads to seizure-like activity with changes in synchrony similar to those seen in the experiments. In the model, a large volley of excitatory synaptic activity from neighboring neurons mediated synchronized onset of bursting. This was further confirmed by the loss of synchrony when the AMPA-type synaptic connections were removed from the network. The reduction in cross-coherence could not be recovered even when the inhibitory connections were made stronger. This arises from the high-frequency nature of spiking activity and the relatively slow nature of GABA_A-type inhibitory synapses.

The computational model illustrates how a small variability in the properties of neurons is sufficient to reduce synchrony if firing at high frequencies, while the low-frequency activity remains highly synchronized during seizure-like events. The change in cross-coherence was observed in a simple conductance-based model, suggesting that these findings are applicable across a wide range of conditions. Furthermore, the model also displayed the distance-dependent effect for cross-coherence seen in experimental data. Taking into account significant variability in intrinsic properties of biological neurons, our study suggests that the loss of synchrony at the high frequency band is an inherent property of neuronal networks where communications between neurons are mediated by synapses and other mechanisms (such as gap junctions) are likely involved in synchronizing neurons at shorter distances.

**Potential mechanisms of high- and low-frequency neuronal synchronization.** Our experimental and computational findings are consistent with previous theoretical studies, which suggest that synchrony of the slow burst event envelope is commonly observed while the spike synchronization within the burst event depends on the connection strength and network topology (Azad and Ashwin 2010; Izhikevich 2001). The synchronization in the network of relaxation oscillators may arise from the fast threshold modulation (Somers and Kopell 1993). Other modes of synchronization have also been shown in Bautin and elliptic bursters (Azad and Ashwin 2010; Izhikevich 2001). However, less is known about necessary and sufficient conditions of spike synchronization within the burst event. In our model we observed spike synchronization within the network of identical neurons, while any small variability led to a loss of synchronization depending on the connectivity strength. Surprisingly, synaptic inhibition, generally a powerful mechanism of neuronal synchronization (Timofeev et al. 2012), was not able to recover synchrony of high-frequency oscillations within an event. Cross-coherence displayed nonlinear dependence on synaptic strength with a peak at the intermediate levels of synaptic inhibition.

Biophysical mechanisms of physiological and pathological synchronization are still under investigation and ongoing discussion. Neuronal synchronization is generally assumed above 100 Hz to depend on synchronous neuronal spiking rather than slow synaptic potentials (Belluscio et al. 2012; Buzsáki 1986; Buzsáki and Silva 2012; Ray and Maunsell 2011). Our data...
suggest that physiological high-frequency activity is better synchronized before epileptic bursts and may therefore mediate epileptiform initiation. Once an epileptiform has started, however, the synchrony of high-frequency oscillations is lost.

Factors affecting the degree of neuronal synchronization during epileptiform activity include changes in extracellular ion concentrations. Indeed, electrical activity of neurons leads to increase of extracellular $K^+$ and decrease of extracellular $Ca^{2+}$. While these local changes are normally compensated by ionic pumps and glial syncitium, significant changes in ion concentrations triggered by electrical activity of one cell may propagate to neighboring neurons, leading to changes in their excitability and therefore contributing to synchronization of electrical activity between neighboring cells (Timofeev et al. 2012). Specifically, this mechanism more likely contributes to the spread and synchronization of neuronal activity during epileptic seizures, when high activity in groups of neurons leads to a large increase in $[K^+]_o$ (Heinemann et al. 1977), whose diffusion increases the excitability of a larger pool of neurons surrounding the epileptic focus (Fröhlich et al. 2010).

This mechanism may lead to an increase in firing rates of neurons synchronously across the network and therefore can promote low-frequency synchronization. At the same time, decrease of extracellular $Ca^{2+}$ and increase in extracellular $K^+$ can decrease synchronization of fast electrical activity by impairing long-range synaptic transmission.

One of our surprising findings was the spatial profile of changes in cross-coherence between all channels at high frequencies. Relatively small clusters of activity around the maxima of the epileptiform increased the degree of synchronization during burst events, which was likely mediated by gap junctions (Anastassiou et al. 2010). There are two types of processes that can lead to initiation of high-frequency activities: first, synchronous population bursting (Bragin et al. 2000; Dzhala and Staley 2004) and second, asynchronous neuronal bursting (Foffani et al. 2007), which results in doubling-tripling frequency harmonics of the original lower frequency. The first process can be described in terms of hypersynchronization of bursting, while the second represents neuronal desynchronization based on reduced spike-timing reliability (Ibarz et al. 2010). Our results suggest that the origin of high-frequency oscillations during epileptiform activity is based on the desynchronization of neuronal spiking, which is reflected in decreased cross-coherence compared with baseline prior to the burst event at a longer distances. In contrast, short-range high-frequency synchronization is enhanced, which is reflected in increased cross-coherence near the focus. Possible mechanisms underlying the decrease in cross-coherence at high frequencies include decreases of $[Ca^{2+}]_o$ mediated by strong epileptiform activity, which diminishes reliability of synaptic transmission and impairs long-range synchronization, or a blockage in axonal spike propagation mediated by membrane depolarization triggered by increase in $[K^+]_o$ (Seigneur and Timofeev 2011). The loss of synaptic synchronization may be compensated at short distances by increased (in conditions of low $[Ca^{2+}]_o$) electrical coupling through gap junctions (Thimm et al. 2005), extracellular field induction (Fröhlich and McCormick 2010), and global fluctuations of extracellular ion concentrations (Heinemann et al. 1977).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: G.P.K. analyzed data; G.P.K., G.F., and M.B. interpreted results of experiments; G.P.K., G.F., and M.B. prepared figures; G.P.K., G.F., and M.B. drafted manuscript; G.P.K. and M.B. edited and revised manuscript; G.F. performed experiments; M.B. conception and design of research; M.B. approved final version of manuscript.

REFERENCES


