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Synchronization of GABAergic Inputs to CA3 Pyramidal Cells Precedes Seizure-Like Event Onset in Juvenile Rat Hippocampal Slices

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Lasztočzi B, Nyitrai G, Héja L, Kardos J. Synchronization of GABAergic inputs to CA3 pyramidal cells precedes seizure-like event onset in juvenile rat hippocampal slices. J Neurophysiol 102: 2538–2553, 2009. First published August 12, 2009; doi:10.1152/jn.91318.2008. Here we address how dynamics of glutamatergic and GABAergic synaptic input to CA3 pyramidal cells contribute to spontaneous emergence and evolution of recurrent seizure-like events (SLEs) in juvenile (P10–13) rat hippocampal slices bathed in low-[Mg\(^{2+}\)] artificial cerebrospinal fluid. In field potential recordings from the CA3 pyramidal layer, a short epoch of high-frequency oscillation (HFO; 400–800 Hz) was observed during the first 10 ms of SLE onset. GABAergic synaptic input currents to CA3 pyramidal cells were synchronized and coincided with HFO, whereas the glutamatergic input lagged by ~10 ms. If the intracellular [Cl\(^{-}\)] remained unperturbed (cell-attached recordings) or was set high with whole cell electrode solution, CA3 pyramidal cell firing peaked with HFO and GABAergic input. By contrast, with low intracellular [Cl\(^{-}\)], spikes of CA3 pyramidal cells lagged behind HFO and GABAergic input. This temporal arrangement of HFO, synaptic input sequence, synchrony of GABAergic currents, and pyramidal cell firing emerged gradually with preictal discharges until the SLE onset. Blockade of GABA\(_{A}\) receptor-mediated currents by picrotoxin reduced the inter-SLE interval and the number of preictal discharges and did not block recurrent SLEs.

Our data suggest that dynamic changes of the functional properties of GABAergic input contribute to ictogenesis and GABAergic and glutamatergic inputs are both excitatory at the instant of SLE onset. At the SLE onset GABAergic input contributes to synchronization and recruitment of pyramidal cells. We conjecture that this network state is reached by an activity-dependent shift in GABA reversal potential during the preictal phase.

INTRODUCTION

Epileptic seizures manifest as uncontrolled limb movements, loss of consciousness and posture, and high-amplitude electroencephalographic (EEG) signals with complex dynamics (Dikanev et al. 2005; Jirsch et al. 2006; Schiff et al. 2000; Worrell et al. 2004). How synchronized activation of large neuronal populations—reflected by the large-amplitude EEG signal of seizures—is brought about is a question of high theoretical and clinical importance. In vivo high-frequency oscillations (HFOs) >200 Hz preferentially occur at the seizure onset (Bragin et al. 2000; Jirsch et al. 2006), a feature manifested in vitro (Dzhala and Staley 2003a; Khoshraftar et al. 2005; Lasztočzi et al. 2004). This temporal alignment of HFOs and transition to seizures suggests a causal link between the two phenomena (Bragin et al. 2000, 2002; Jirsch et al. 2006; Lasztočzi et al. 2004). HFOs are linked to hyperexcitability and synchrony of neuronal networks, the two hallmarks of epileptiform activity (Le Van Quyen et al. 2006; McCormick and Contreras 2001; Perez Velazquez and Carlen 2000; Traub et al. 2001). Hyperexcitable state, which renders the network prone to spontaneous seizures, may result from disruption of balance between excitation and inhibition. This could be either a consequence of alterations in glutamatergic and/or GABAergic synaptic input per se (Cossart et al. 2001; Epsztein et al. 2006; Trotter et al. 2006) or a positive shift in the reversal potential of GABA\(_{A}\) receptor-mediated currents (Cohen et al. 2002; Dzhala and Staley 2003b; Fujiwara-Tsumakimoto et al. 2007; Khazipov et al. 2004; Köhling et al. 2000; Lopantsev and Avoli 1998; Perez Velazquez 2003).

Excitatory action of the inhibitory transmitter amino acid GABA has been suggested to be responsible for increased seizure susceptibility of an immature brain (Dzhala and Staley 2003b; Dzhala et al. 2005; Khazipov et al. 2004; Tyzio et al. 2007). Although the direct excitatory action of GABA has been demonstrated in the neonatal brain (postnatal days <8) (Khazipov et al. 2004; Rivera et al. 1999; Tyzio et al. 2007), other more complex ways of GABAergic excitation have been established in adult animals and in human epileptic brain (Cohen et al. 2002; Epsztein et al. 2006; Fujiwara-Tsumakimoto et al. 2003, 2007; Kaila et al. 1997; Lamsa and Kaila 1997; Marty and Llano 2005; Perez Velazquez 2003; Staley et al. 1995). Transitory between neonatal and adult, slices from juvenile (P10–13) rat hippocampus are frequently used in experimental epilepsy research as they are more susceptible to develop seizures than slices from adults (Heinemann et al. 1991; Köhling et al. 2000; Lasztočzi et al. 2004). Interestingly, experimental data supported either excitatory (Dzhala and Staley 2003b; Khazipov et al. 2004) or inhibitory/shunting (Rivera et al. 1999; Tyzio et al. 2007) actions of GABAergic transmission at this age. Although the susceptibility to seizures of the juvenile brain is well documented, information on the short-term dynamics of glutamatergic and/or GABAergic synaptic drive contributing to ictogenesis is much more scarce.

Juvenile rat hippocampal slices bathed in low-[Mg\(^{2+}\)] artificial cerebrospinal fluid (ACSF) express preictal discharge trains with increasing similarity to SLE onset, confinement of field potential (fp) HFOs (400–800 Hz) to the start of discharges, and gradual emergence of synchronized synaptic currents onto CA3 pyramidal cells (Lasztočzi et al. 2004). Although the contribution of these synchronized inputs to SLE initiation was documented (Lasztočzi et al. 2004), the relative weight of GABAergic versus glutamatergic components has...
not been established. Glutamatergic (Dzhala and Staley 2004; Palva et al. 2000) and GABAergic (Jones and Barth 2002; Khalilov et al. 2005; Palva et al. 2000) synaptic interactions were implicated in the generation of HFOs and seizure-related synchrony. Accomplished by a detailed analysis of temporal relationships between pyramidal cell firing, fp activity and synaptic input sequences during SLEs, the aim of the present study was to dissect GABAergic and glutamatergic contributions to SLE dynamics.

**M E T H O D S**

Animals were kept and used in accordance with the European Council Directive of 24 November 1986 (86/609/EEC) and the Hungarian Animal Act 1998 and associated guidelines. All efforts were made to reduce animal suffering and the number of animals used. Transverse, 400-µm-thick hippocampal-entorhinal slices were cut in horizontal plane from brains of juvenile (P10-13) male Wistar rats (Toxicoop, Budapest, Hungary) as described elsewhere (Lasztóczi et al. 2004). Slices were put in a submerge-type recording chamber continuously perfused by ACSF at 36°C (composition in mM: 124 NaCl, 10 glucose, 3 KCl, 1.25 NaH2PO4, 2 MgSO4, 2 CaCl2, and 26 NaHCO3; saturated with carbogen gas). To evoke spontaneous recurrent SLEs, the perfusing solution was switched to one with no added Mg2+ ions and [K+] raised to 5 mM (low-[Mg2+] ACSF). In a subset of experiments, the CA3 region was isolated by knife cuts after the SLE activity has been established.

Recordings were performed as described previously (Lasztóczi et al. 2004) with minor modifications. Signals obtained by Multiclamp700A amplifier (Axon Instruments, Foster City, CA) were low-pass filtered at 2 kHz and digitized at 10 kHz (Digidata1320A, Axon Instruments). For single-cell recording, CA3 pyramidal cells were identified visually. Extracellular (2–5 MΩ) and cell-attached (5–9 MΩ) electrodes were filled with ACSF. For cell-attached recordings, holding potential was set to −70 mV. Experiments were discarded if signs of partial “break-in” had occurred [e.g., spontaneous postsynaptic currents (sPSC) appeared]. For voltage clamp, pipettes (4–6 MΩ) were filled with a solution containing (in mM) 130 CsMeSO4, 10 NaCl, 0.3 CaCl2, 2 ATP (magnesium salt), 1 EGTA, and 10 HEPES (pH set to 7.3 with 1 N CsOH). To suppress escape-action currents, 5 mM QX 314 (Tocris, Bristol, UK) was added. Cells were voltage-clamped around 0 mV (at −5, 0, or +5 mV), −45 mV (at −40, −45, or −50 mV), or −30 mV (−25, −30, or −35 mV) to record GABAergic (outward), glutamatergic (inward), or combined (outward-inward) currents, respectively. The holding potential was adjusted in individual experiments to a level where outward or inward sPSCs (as appropriate) just disappeared. These holding potential values were in agreement with the reversal potentials experimentally determined for pharmacologically isolated spontaneous GABAergic and glutamatergic sPSCs (see RESULTS). If signs of seal deterioration (large sustained inward currents with increased noise) or cell closure (reduction of sPSC amplitude associated with slowing of their dynamics and a shift in the holding current toward 0) occurred, the recordings were discarded from analysis. For current clamp with low-[Cl−] intracellular solution, pipettes (3–5 MΩ) contained in (mM) 135 KGlucuronate, 10 NaCl, 0.05 CaCl2, 2 ATP (magnesium salt), 1 EGTA, and 10 HEPES (pH set to 7.3 with 1 N KOH). In the case of high-[Cl−] condition, intracellular solutions [KGlucuronate] = 85 mM and [KCl] = 50 mM were applied with all other concentrations unchanged. To explore cellular responses, we applied hyperpolarizing (30–100 pA) and depolarizing (30–200 pA) current steps. In some cases, ±50- or 100-pA current steps were also applied while recording sPSCs under current clamp, but this did not affect spike timing, and therefore data were pooled. To determine the reversal potential of synaptic currents, pharmacologically isolated miniature excitatory postsynaptic currents (mEPSCs) or miniature inhibitory postsynaptic currents (mIPSCs) were recorded in the presence of 1 µM tetrodotoxin and 100 µM picrotoxin or 1 µM tetrodotoxin, 10 µM 6-cyano-7-nitroquinolinolene-2,3-dione (CNQX), and 50 µM dL 2-amino-phosphopentonic acid (dL AP-5), respectively while the holding potential was changed between −80 and +80 mV in 20-mV steps. Linear function (Origin 6.1) has been used to fit the data: The value obtained for 0 pA current with the standard error of the fit was defined as the reversal potential. Voltages recorded or commanded in whole cell configuration were subject to a junction potential correction of 15 mV calculated with the built-in application of Clampfit, based on the composition of intra- and extracellular solution.

Two to 10 SLEs were analyzed from each slice. Being not fully developed, first SLEs were discarded. SLE intensity was calculated as the SD of high-pass filtered (>1 Hz) fp trace containing the whole SLE, normalized to the SD of preceding period, and when <200%, SLEs were discarded due to the inability to unequivocally define event timing. Preictal discharges were numbered with negative numbers starting from the SLE onset backward (e.g., p-1, p-2, p-3, etc.). Due to different numbers (4.3 on average) of preictal discharges, N decreased for preictal discharges more distant from the SLE onset. For quantitative analyses of preictal trends, p-5 to p-1 and SLE onset were included.

Whenever possible, we identified the start of fp discharges by the peak of negative deflection (Lasztóczi 2004). If this was missing, start points were identified visually with the experimenter blind to the contents of the other channel. The relationship between the fp discharges and spike timing of pyramidal cells was explored by constructing spike probability histograms within a 200-ms window centered at the start of fp discharges. Spike peak times were identified semiautomatically (MiniAnalysis, Synaptosoft, Decatur, GA) and inspected visually. Amplitude threshold for action potential and action current detection were set to 17–20 mV and 13–20 pA, respectively. Partial spikes (if 10–90% slope >2.5 mV/ms) were not discriminated from full spikes. We constructed separate spike density histograms for 10 different discharge categories (preictal discharges p-5, p-4, p-3, p-2, and p-1, secondary preictal discharges, SLE onsets, tonic discharges, clonic discharges, and secondary clonic discharges) by pooling all the relative spike time data for all discharges of all SLEs within a category. To make sure that one discharge contributes to a particular bin with a maximum of one spike, we chose a 3-ms bin size, allowing us to calculate the spike probability histograms by simply normalizing the spike density histograms to the number of discharges within a category. Histograms were fitted with built-in Origin 6.1 functions (OriginLab, Northampton, MA). Gaussian model was better for tonic and secondary discharges, while the skewed Extreme model was better for others. All histograms were fitted first with one and then with the sum of two functions. Multiple peaks were accepted if resulted in an increase in R2 of >0.01. The smaller peaks at the extremes of histogram windows in case of secondary and tonic discharges represent spikes associated with neighboring discharges. Therefore—although two to three component fits accounted for their presence—values are reported for the relevant component only.

For discharge-associated outward and inward current transients (compound postsynaptic currents; comPSC) amplitudes and 10–90% current rise times were measured using the Mini Analysis software. To analyze changes in current parameters within the SLE, we used a time normalization protocol separately for the tonic and the clonic periods. For this, we divided both periods into six equal-length epochs (epochs 1–6 for both tonic and clonic periods in Fig. 4), first averaged and aligned with the standard error of the fit was defined as the reversal potential. Voltages recorded or commanded in whole cell configuration were subject to a junction potential correction of 15 mV calculated with the built-in application of Clampfit, based on the composition of intra- and extracellular solution.

For temporal analysis of synaptic input sequences, 200-ms current traces centered at the start of fp discharge were extracted. These were scaled between their minima and maxima, and averaged across discharges. This analysis was performed on representative SLEs (1 for outward and 1 for inward if available) from each slice. Due to the plateau-like nature of glutamatergic comPSCs under some conditions, for the purpose of display on Fig. 8, time of occurrence of GABAergic synchrony and firing during seizures
and glutamatergic currents were defined by the time when currents reached 90% of the maximum in the analysis window.

To extract the intensity of high-frequency fp activity with temporal resolution comparable to other analyses, we transformed fp traces containing SLEs into 400- to 800-Hz intensity traces with a sliding window approach. To avoid phase shifts introduced by most filters, we band-pass filtered the down-sampled (5 kHz) SLE-containing traces by calculating the difference between a low-pass filtered (<800 Hz) and a high-pass filtered (>400 Hz) trace. We used Gaussian type digital filters (pClamp9) as they do not introduce phase shifts. The resulting trace was transformed into an intensity trace by calculating the SD of the trace with a continuously sliding 3.2-ms window. Epochs (200 ms) centered at the fp discharge start were finally extracted and averaged across different SLEs. This analysis was performed on one representative SLE for each slice.

Unless stated otherwise in the text, one-way ANOVA followed by Bonferroni’s multiple post hoc comparison was used for statistical testing with P < 0.05 considered significant, and data are reported as means ± SD. Data processing, analysis, and graphical representations were executed with pClamp9 (Axon Instruments), Origin 6.1 (OriginLab), MiniAnalysis (Synaptosoft), Corel9 (Corel, Dallas, TX) software, and self-devised scripts in MATLAB 6.5 (MathWorks, Natick, MA) environment.

RESULTS

In 76% of juvenile (P10-13) rat hippocampal slices, perfusion with low-[Mg$^{2+}$] ACSF resulted in recurrent SLEs with 760 ± 230 s latency, 82 ± 28 s length, and 335 ± 133 s inter-SLE interval (Fig. 1A; n = 70 slices from 38 animals) (Anderson et al. 1986; Walther et al. 1986). First signs of forthcoming SLEs were the appearance of large-amplitude (>0.5 mV) discharges that consisted of 0.1- to 0.3-s positive shift with fast (>100 Hz) oscillation superimposed (Fig. 1B; preictal discharges). Further discharges of the preictal period (there was 4.3 ± 2.8 on average) showed gradual build-up until the SLE onset (Fig. 1B) (Khoshravani et al. 2005; Laszto´czi et al. 2004). A decelerating train of tonic discharges followed the SLE onset with the tonic-to-clonic transition marked by the reappearance of secondary discharges (Fig. 1B).

To study temporal relationship between cellular events and fp, these should be recorded sufficiently close. Dual extracellular recordings in CA3 addressed this issue, with the distance between electrodes ranging from 26 to 895 μm (n = 19 slices from 15 animals). Delay histograms of tonic, clonic, and secondary clonic discharges peaked at 0 ms for distances <100 μm (n = 7 slices), whereas nonzero peaks were frequently found for distances >100 μm. Therefore in parallel single-cell and fp recordings, we placed the two electrodes within 100 μm.

Spikes coincide with the start of fp discharges: cell-attached recordings

We first addressed the spike timing of CA3 pyramidal cells in relation to epileptiform fp discharges. To keep the intracellular milieu as intact as possible and yet to record spike timing accurately, we recorded action currents of CA3 pyramidal cells in cell-attached voltage-clamp configuration (8 cells from 7 animals) (Perkins 2006). Before switching the perfusion to low-[Mg$^{2+}$] ACSF, cells emitted action currents at a low overall rate (0.59 ± 0.84 Hz) typically in short bursts of two to six spikes (intraburst frequency: 153 ± 58 Hz), identifying the recorded cells as pyramidal cells. In low-[Mg$^{2+}$] ACSF, action current rate increased to 3.50 ± 2.54 Hz indicative of network activation. During SLEs, we found action currents associated with preictal discharges, SLE onsets and tonic and clonic discharges (Fig. 2, A and B; n = 30 SLEs from 8 slices of 7 animals). Maximum instantaneous action current frequency during SLEs (including the preictal period) was 230 ± 39.1 Hz with interspike intervals no shorter than 3.1 ms observed. Accordingly, binning histograms at 3 ms allowed us to accurately estimate spike probability without including adjacent spikes in any bin. The fp discharge start times were identified either by the minimum of negative deflection at their start or manually as appropriate (Laszto´czi et al. 2004) (see METHODS for details). This method has a high (ms range) accuracy for all the discharges starting with a negative fp deflection (late preictal discharges, SLE onset, tonic and clonic discharges) (see Laszto´czi et al. 2004) and thus allows the temporal alignment of measures reflecting different aspects of network behavior [reported as distributions for spike timing and mean current or intensity (± SE) for synaptic input and HFO].

Action currents were associated with the rising and sustained phases of early preictal discharges (p-5 in Fig. 2C). This was reflected by the spike probability histogram (plot “p-5” in Fig. 2D; pooled for 6 SLEs from 5 slices) well described with a single-component curve with a peak at +42 ms (black line on Fig. 2D; see METHODS for the details on fitting procedures). SLE onsets displayed markedly different pyramidal cell firing probability distributions (Fig. 2, C and D, plot for SLE onset from 30 SLEs from 8 slices) with an earlier and sharper peak in
addition to the more delayed one. Indeed this histogram was better fit with the sum of two components (see METHODS for details) with peaks at $H_{11001}^{2.8}$ and $H_{11001}^{51}$ ms (black lines on Fig. 2D). In parallel to the evolution of fp waveform (Fig. 1B), spike probability histograms showed gradual transformation between p-5 and SLE onset (Fig. 2, C and D, pooled for 13, 17, 25, and 30 SLEs from 7, 7, 8, and 8 slices, from p-4 to p-1, respectively). Contrasting the relatively constant peak times (around 40 ms) and amplitudes of the slower component throughout the preictal period, the sharper and earlier component appeared at p-2 only and had increasing amplitude and accelerating peak toward the SLE onset (Fig. 2D: black lines).
We frequently observed action currents aligned to the negative fp deflections marking the start of fp events. In agreement with this observation, the spike probabilities of the histogram zero bins were large throughout the preictal period (Fig. 2D; Table 1). This tendency was most obvious for the SLE onset where the firing probability was eventually at its maximum level within the zero bin (Fig. 2D; see C also).

Pyramidal cell firing was associated with the start of tonic (Fig. 2C, middle), primary clonic (Fig. 2C, right), secondary preictal, and secondary clonic discharges as well. For these discharge types, spike probability histograms (pooled for 30 SLEs from 8 slices) peaked with high values at 0 ms and accordingly were fitted with curves peaking close to zero. Histogram for primary clonic discharges and the curve fitted to it were much like the histogram and the curve of SLE onset (Fig. 2D; slow and fast components peaked at +41 and +0.9 ms, respectively).

**Dynamics of synchronized GABAergic input: voltage-clamp recordings**

The preceding data indicate that during the preictal period CA3 network gradually enters a state characterized by synchronous and accelerated action potential firing timed to the start of discharges. The question arises if this can be explained by the temporal organization of synaptic inputs to individual CA3 pyramidal cells. To address this, we performed whole cell voltage-clamp recordings from CA3 pyramidal cells with CsMeSO₃ pipette solution during recurrent SLEs. In a set of pilot experiments, we recorded pharmacologically isolated mEPSCs and mIPSCs (in the presence of 1 μM tetrodotoxin and 100 μM picrotoxin or 1 μM tetrodotoxin, 10 μM CNQX, and 50 μM d-AP-5, respectively) and determined their reversal potentials under control conditions. Recording currents at EPSC reversal potential would reflect dynamics of GABAergic currents (and vice versa) even in the absence of pharmacological receptor blockade (Trevelyan et al. 2006). The reversal potential of mIPSCs was −48 ± 1.6 mV and that of mEPSCs was −5 ± 2.8 mV (n = 6 cells from 3 animals and n = 4 cells from 3 animals for IPSCs and EPSCs, respectively). During low-[Mg²⁺]-induced recurrent SLEs, cells were held either close to 0 mV (n = 23 SLEs from 10 cells of 5 animals) or to −45 mV (n = 17 SLEs from 8 cells of 5 animals) to record GABAergic (outward) and glutamatergic (inward) currents, respectively (Figs. 3A and 4A). To record the two components as separately as possible, the exact holding potential for each experiment was determined by adjusting it in 5-mV steps to a level where outward or inward sPSCs (as appropriate) just disappeared.

fp discharge-associated inward and outward current transients resulted from temporal summation of individual postsynaptic currents (Fig. 3C, open arrow on clonic trace), as confirmed by their high-amplitude, slow rise-time and rugged, uneven onset (Figs. 3, A–C, and 4A). These are hereby referred to as compound postsynaptic currents (comPSC). Their 10–90% rise time therefore estimates the synchrony of underlying individual PSCs (Lasztczi et al. 2004) (faster rise indicates higher synchrony). During the preictal period (from p-5 to SLE onset), inward comPSC rising did not change (Fig. 3B). The 10–90% current rise times for discharges p-5 to SLE onset were 48 ± 20, 55 ± 14, 60 ± 17, 49 ± 15, 53 ± 15, and 38 ± 22 ms (n = 6, 9, 10, 14, 17, and 17 SLEs from 3, 5, 6, 8, 8, and 8 slices, respectively; P = 0.04 by ANOVA, but no significant differences within the preictal period with Bonferroni’s post hoc test; Fig. 3D, gray circles). By contrast, when cells held...
close to 0 mV, marked changes were observed in outward (GABAergic) current waveforms. The rising phase of comPSCs associated with early preictal discharges was slow (rise times frequently >100 ms), whereas comPSCs associated with late preictal discharges and SLE onsets rose fast with sharp upstroke (rise times frequently <20 ms; Fig. 3C). Mean rise times from p-5 to SLE onset showed a decreasing trend and were 208 ± 167, 142 ± 119, 184 ± 193, 136 ± 129, 50 ± 61, and 29 ± 24 ms, respectively (Fig. 3D, black circles; n = 5, 7, 14, 22, 23, and 23 SLEs from 4, 5, 7, 10, 10, and 10 slices, respectively.)
respective). The differences within outward comPSC rise times were highly significant (P < 0.05 for differences p-5 to p-1 and p-5, p-4, p-3, and p-2 to SLE onset; ANOVA followed by Bonferroni’s post hoc test). Amplitudes were normalized to average preictal period values (including SLE onset) that amounted to 1,116 ± 446 pA and 1,108 ± 458 pA (outward and inward, respectively). Amplitude of glutamatergic currents showed mild increasing trend during the preictal period (Fig. 3E, gray circles), but this reached significant levels only between p-5 and p-2, whereas outward currents showed no change but a significant drop to 76 ± 20% at SLE onset (Fig. 3E, black circles; P < 0.05). This drop may in part result from SLE onsets frequently starting from an elevated baseline (residual current from the last comPSC; see Fig. 4B and DISCUSSION).

Entering the tonic period there was a further substantial drop in GABAergic comPSC amplitude (to ~10%), followed by a gradual increase, regaining the original (preictal period) amplitude at SLE end (Fig. 3, A and E, black circles; P < 0.05). In spite of increasing tendency, rise time of the outward comPSCs remained reduced from the SLE onset onwards (no further significant differences; Fig. 3 C and D, black circles). The glutamatergic inward comPSCs showed decreased amplitude and rise time from the start of the tonic phase (P < 0.05; Fig. 3E). The amplitude remained low for the rest of SLE (no further significant change), but the rise time gradually regained its original value during the clonic phase (Fig. 3, B and D, gray circles; P < 0.05). The temporal sequence of synaptic inputs was explored by time aligning current recordings from CA3 pyramidal cells to the start of preictal, tonic, and clonic fp discharges. At holding potentials close to 0 mV (Fig. 4, A–C; n = 10 SLEs) substantial outward currents were present already before and at the start of preictal discharges and SLE onsets (Fig. 4C). We quantified the synaptic input at discharge start by the mean current found in the zero 3-ms bin (e.g., from –1.5 to +1.5 ms). This was 715 ± 422, 596 ± 235, 683 ± 270, 729 ± 396, 855 ± 318, and 620 ± 336 pA for GABAergic currents of discharges p-5 to SLE onset, respectively (n = 3, 4, 6, 10, 10, and 10 SLEs, respectively; Fig. 4E, top). Inward comPSCs of preictal discharges and SLE onsets (cells clamped close to −45 mV) started at or after the start of the fp discharge (Fig. 4, A–C) and thus displayed small zero bin currents (55.9 ± 16.3, 14.4 ± 2.15, 64.5 ± 37.4, 53.5 ± 44.5, 82.6 ± 106, and 135 ± 83.5 pA from p-5 to SLE onset, respectively; n = 3, 4, 4, 8, 8, and 8 SLEs, respectively; Fig. 4E, top). Although the driving forces for the two types of comPSCs should be similar when recorded at the reversal potential of the other (they are at exactly the same distance from their reversal potential) other sources of variability could also affect these measurements. To assess the temporal organization of synaptic inputs with the exception of confounding amplitude changes, the current transients were scaled between the minimum and maximum found in the ±100-ms window, and the scaled currents were averaged across SLEs, giving fractional currents for the period sampled (Fig. 4D). A progressive increase in the fractional zero bin (−1.5 to 1.5 ms) outward currents was observed from p-5 to SLE onset (Fig. 4E, bottom), while the peaks of the average currents shifted closer to discharge start (+44.9, +66.2, +31.8, +14.5, +7.6, and +4.4 ms from p-5 to SLE onset, respectively; exemplified with p-2 and SLE onset traces displayed in Fig. 4, C and D). Contrasting the outward currents, fractional inward currents were small within the zero bin throughout the preictal period (Fig. 4E, bottom). Moreover, instead of displaying clear peaks, average glutamatergic currents often did not start to decay within the analysis window (Fig. 4, C and D). Statistical analysis disclosed significant differences between zero bin fractional inward and outward currents for all discharge types. Within inward and outward current group, comparisons showed significant differences between outward fractional currents of p-4 and SLE onset only (P < 0.05).

Similarly to the SLE onset, the scaled and averaged GABAergic outward current of the tonic and clonic discharges peaked close to the discharge start (at +0.4 and +2.6 ms, respectively) and displayed large fractional currents within the zero bin not significantly different from the SLE onset. The peak of the average inward current associated with tonic discharges (Fig. 4D) at +13 ms indicated that the glutamatergic component lagged behind the start of discharge and the GABAergic current. Indeed fractional zero bin inward currents were significantly smaller than the respective fractional outward currents (P < 0.05).

These data brought to surface a clear temporal sequence of synaptic events associated with fp discharges: a transient and highly synchronized GABAergic input followed by a sustained glutamatergic input. To further test the validity of this scenario, we recorded a combination (−sum) of GABAergic and glutamatergic currents during SLEs by voltage clamping CA3 pyramidal cells to holding potential halfway between the reversal potentials for GABAergic and glutamatergic currents (around ~30 mV; n = 9 SLEs from 5 cells; Fig. 4, A–C). These recordings showed that the preictal discharges, the SLE onsets (except one with no discernible GABAergic transient) and the clonic discharges started with an outward current coincident with the start of fp discharges, and only later the inward component prevailed (Fig. 4, B and C). This is exactly what one would expect if the scenario outlined above were valid. The outward component associated with the start of tonic discharges was much smaller if discernible at all (Fig. 4C).

Pyramidal cell spike-timing depends on intracellular [Cl−]: current-clamp recordings

The cell-attached recordings disclosed pronounced synchronization and intense firing of CA3 pyramidal cells in a narrow time window that is dominated by GABAergic input. One possible explanation for this paradox finding is that at the start of some discharges the inhibition provided by GABAergic input is diminished or GABAergic currents are functionally excitatory due to a shift in [Cl−]/HCO3− distribution. To explore how changes in GABAergic function would affect the spike timing, we recorded and compared the timing of fp discharge-associated spikes from CA3 pyramidal cells in whole cell current-clamp mode, with low-[Cl−] (10 mM) and high-[Cl−] (60 mM) intracellular solutions. Determined for pharmacologically isolated mPSC under control conditions, reversal potential of GABAergic currents changed from −65 ± 1.7 mV (low-[Cl−]; n = 4 cells from 3 animals) to −42 ± 1.8 mV (high-[Cl−]; n = 5 cells from 3 animals) between the two intracellular solutions. Depending on the validity of our hypothesis forcing the GABAergic input hyper- or depolarizing
with setting the intracellular [Cl\(^-\)] low or high should result in differential spike timing at the start of discharges.

With the low-[Cl\(^-\)] solution in pipettes (n = 12 cells from 8 animals), cells had resting membrane potential and input resistance of \(-79 \pm 8.6\) mV and \(189 \pm 66.0\) M\(\Omega\), respectively. Depolarizing current injections evoked a train of overshooting action potentials, starting with intrinsic burst in nine cells. Ten of the recorded cells were filled with AlexaFluor 488 (100–200 \(\mu\)M) and observed under confocal microscope. They showed morphology characteristic of pyramidal cells (data not shown). Perfusion of slices with low-[Mg\(^{2+}\)] ACSF resulted in the depolarization of cells to \(-72 \pm 3.0\) mV (\(P < 0.05\); paired \(t\)-test; measured before SLEs). SLEs (n = 37, from 12 cells of 8 animals) were associated with slowly decaying plateau-like depolarization to \(-50 \pm 4.0\) mV (Fig. 5A), and were followed by membrane depolarization.

**Fig. 5.** Spike timing of current-clamped pyramidal cells during SLEs. A and B: representative fp (gray traces) and current-clamp (black traces) recordings with low 10 mM [Cl\(^-\)] (A) and high 60 mM [Cl\(^-\)] (B) pipette solution of individual discharges time-aligned to the start of the fp discharges (vertical dashed lines). Left: preictal discharges and the SLE onset (bottom traces) are displayed. Five representative tonic and 5 representative clonic discharges are overlaid in middle and right plots, respectively. C and D: spike probability histograms for different discharge categories as indicated above the plots. The starts of individual fp discharges were assigned as the 0 time point (vertical dashed lines). Spike outputs were pooled into 3-ms bins from all discharges available. Black traces represent fit components and their sum.
potentials significantly more negative than before SLEs ($-77 \pm 4.8$ mV; $P < 0.05$, paired $t$-test). Instantaneous action potential frequencies were always $<170$ Hz.

Preictal discharges and SLE onsets were accompanied with depolarizations and action potential firing (Fig. 5, A and C). The spike probability histogram of early preictal discharges showed that pyramidal cells did not fire before or at the start of the event, but firing probability smoothly increased during the first $40–50$ ms of the discharge (plot “p-4” in Fig. 5C; pooled for 27 SLEs from 7 animals). The firing pattern associated with SLE onset was markedly different (Fig. 5A and plot for the SLE onset in C; pooled for 37 SLE onsets from 8 slices). Firing probability remained low (0.08) at the start of fp discharge but increased sharply shortly afterward and reached peak (0.41) at the $+6$ ms bin. This pattern was adequately described by the sum of two components (see METHODS): a sharper and earlier, peaking at $+4.6$ ms and a wider and later peaking at $+19$ ms. Spike probability histograms of preictal discharges (plots “p-4” to “p-1” in Fig. 5C; pooled for 27, 34, 36, and 37 discharges of 7, 7, 7, and 8 animals, respectively) disclosed the evolution of this pattern with increasing and accelerating fast fit component (peak times of $+15$, $+16$, $+10$, and $+4.9$ ms for p-4 to p-1 discharges, respectively; Fig. 5C). Spike probability in zero bins was relatively low throughout the preictal period and the SLE onset indicating that not much firing was associated with the start of discharges (Fig. 5, A and C). Clonic discharges were also associated with depolarizing potentials and action potential firing (Fig. 5, A and C). The overall firing pattern associated with clonic discharges was similar to that of the SLE onset (Fig. 5, A and C, pooled for 37 SLEs from 12 cells of 8 animals). As indicated by the low value of firing probability at the zero bin, spikes rarely timed to the start of discharge but were synchronized to the $+9$ ms bin (Fig. 5, A and C). Indeed in several cases, clonic discharges and SLE onsets started with a short hyperpolarization under whole cell conditions (data not shown), thus displaying clear inhibition during a time period when cells recorded in cell-attached mode showed peak firing. Tonic discharges of the first few seconds, as well as secondary preictal and secondary clonic discharges often lacked action potentials probably as a consequence of depolarization-induced inactivation (Ziburkus et al. 2006). The histograms of tonic (Fig. 5C; pooled for 37 SLEs from 12 cells of 8 animals), secondary preictal, and secondary clonic (not shown) discharges had peaks lagging behind start of fp discharge (Fig. 5C). Noticeably, the spike probabilities in zero bin were much lower than peak values.

We also recorded CA3 pyramidal cells filled with high-[Cl$^-$] intracellular solution that made GABAergic inputs depolarizing (see preceding text; $n = 11$ cells from 6 animals, Fig. 5, B and D). Resting potential of the cells was $-73 \pm 7$ mV, which increased to $-60 \pm 6.6$ mV after changing the perfusion solution to low-[Mg$^{2+}$] ACSF. In all cases, rhythmic membrane depolarizations (of $24 \pm 12$ mV amplitude) associated with action potential bursts appeared after a few minutes in low-[Mg$^{2+}$] ACSF. Membrane potential changes associated with SLEs were similar to those recorded from cells filled with low-[Cl$^-$] intracellular solution (plateau like depolarization to $-44 \pm 4$ mV, and hyperpolarization to $-79 \pm 5$ mV after SLEs).

Preictal discharges (p-4, p-3, p-2, p-1) and SLE onsets (pooled for 6, 9, 11, 13 SLEs from 4, 5, 5, 5 animals, respectively) were accompanied with depolarizations and action potential firing (Fig. 5, B and D). However, in contrast to the results obtained with low-[Cl$^-$] intracellular solution, the spike probability histogram of preictal discharges p-4 to p-1 showed that pyramidal cells often fire before or at the start of the event, but less after that (Fig. 5, C and D). Cell firing associated with SLE onset showed a different pattern. Spike probability peaked in the $-3$-ms bin and was smaller but still high in the 0-ms bin. Spiking activity—just like in case of preictal discharges—decreased following the discharge start (Fig. 5, B and D). Clonic discharges were also associated with depolarizing potentials and action potential firing (Fig. 5, B and D pooled for 16 SLEs from 11 cells of 6 animals). Firing probability started to increase and actually reached its peak (at $-3$ ms) before the discharge start, remained high in the 0-ms bin and dropped sharply in the forthcoming bins. This pattern was described by the sum of two components: a sharper, peaking at $-1.8$ ms and a wider and smaller peaking at $-5.8$ ms (due to the small number of action potentials, we did not use fits to analyze preictal discharges and SLE onsets for the high-[Cl$^-$] intracellular solution). The high firing probability preceding the preictal, SLE onset and clonic discharges (Fig. 5, B and D) can be explained by the intense (and in this case excitatory) GABAergic input preceding these discharges (data not shown, but see Figs. 3C and 4C), while the silencing after the discharge start is most likely the consequence of inactivation caused by the joint action of glutamatergic and depolarizing GABAergic inputs. Under high-[Cl$^-$] conditions, GABAergic excitation may contribute to depolarization-induced block of action potentials during the tonic phase as well, as firing probability associated with tonic discharges (Fig. 5, B and D, pooled for 16 SLEs from 11 cells of 6 animals) was low but precisely timed to the start of the discharge when compared with the low-[Cl$^-$] situation (compare Fig. 5, D to C).

These data show that the spike timing of CA3 pyramidal cells at the start of SLE onset and clonic discharges is dependent on intracellular [Cl$^-$] and is thus critically regulated by GABAergic inputs. Because under undisturbed intracellular conditions (cell-attached measurements) the spike timing at these SLE stages resembled the spike timing observed with high-[Cl$^-$] rather than low-[Cl$^-$] intracellular solution, our data also imply a relatively high GABA reversal potential at the SLE onset and throughout the SLE.

**HFOs at SLE onset are confined to the start of discharge**

Although 400–800 Hz fp activity was present during interictal periods as well, large increases in this band were associated with preictal discharges, SLE onsets, and clonic discharges. ($n = 70$ SLEs from 70 slices of 38 animals, Fig. 6A; see METHODS). At SLE onsets, clonic discharges and some late preictal discharges HFOs appeared as short ($5–20$ ms) epochs of fp activity confined to the start of these discharges (Fig. 6B) (Lasztoczi et al. 2004). Sometimes—especially at SLE onsets—these HFOs were composed of only a few cycles. Temporal patterns of HFOs were investigated by averaging the HFO intensity traces for distinct
discharge categories across different SLEs with the start of fp discharge assigned as the zero time point (Fig. 6C). For early preictal discharges (see the plot “p-5” on Fig. 6C; pooled for 23 SLEs) large HFO intensities were distributed across long periods (~100 ms) mainly after the start of the discharge. Only minor changes in temporal HFO intensity distribution were observed for the next preictal discharges (p-4 to p-1; n = 32, 47, 60, and 69 SLEs, respectively; Fig. 6C), although at p-1, a peak located very close to the start of the discharge (at +0.8 ms) emerged. The HFO intensity distributions for SLE onset (n = 70 SLEs) and clonic discharges (n = 70 SLEs) displayed much clearer peaks again very close to the start of discharge (both at +0.4 ms). HFO intensity had peaks associated with the start of tonic and secondary discharges (0 ms; tonic; pooled for 70 SLEs) or very close to those (at +0.6 and +0.4 ms, for secondary preictal and secondary clonic discharges, respectively). These data identify a short epoch of HFO in the 400- to

**FIG. 6.** Temporal patterns of high-frequency oscillations (HFOs) during SLEs. A: fp recording (top trace) of an SLE from the CA3 pyramidal layer, the trace band-pass filtered between 400 and 800 Hz (middle trace), and fp intensity in 400- to 800-Hz band integrated with a 3.2-ms sliding window (bottom trace). Open and filled arrowheads point to preictal discharges and SLE onset, respectively. The arrow marks the position of the tonic-to-clonic transition. B: the SLE onset and a clonic discharge enlarged from the SLE shown in A. The traces are as in A. The vertical dashed lines mark the discharge start. C: average intensities of activity in 400- to 800-Hz band during different discharge categories (as indicated above the plots). The gray shaded areas represent the ±SE ranges. The vertical dashed lines mark the point of alignment (discharge start).
GABA receptor-dependent activity is present at the SLE onset and during subsequent SLE periods (Lasztoczi et al., 2004).

**SLEs are generated within the network of the CA3 region**

Trevelyan et al. (2006) described synaptic input sequences resulting from discharge spread in the neocortex. To interpret our data, it is essential to know if SLEs (and individual discharges within) are generated locally or the CA3 network is driven by synchronized input. When the CA3 region was isolated from other hippocampal regions by a knife cut (n = 4 slices from 4 animals) after the third SLE had occurred, subsequent to a 10- to 15-min-long silent period SLEs reappeared in the CA3 region in three of four slices. This proves that the isolated CA3 is able to generate and support epileptiform activity, but does it generate SLEs in the intact slice preparation as well? To answer this question, we mapped potential sources of synchronized input to the CA3 with double extracellular recordings. Concurrently with large-amplitude (>1 mV) SLEs in the CA3, only tiny (usually <0.2 mV) f.p. fluctuations (4 slices) or no activity (2 slices) were detected in the granule cell layer of the dentate gyrus. Recordings from layer II/III of the entorhinal cortex (n = 8 slices), disclosed epileptiform activity that was temporally uncoupled from the activity in the CA3 in six slices and SLEs delayed in the entorhinal cortex by 16–47 s in two slices. Even if the SLE activity was overlapping in the two regions, individual discharges showed no temporal correlation. SLEs in the CA3 region had always their counterparts in the CA1 region (n = 8 slices). The delay (spread time from CA3 to CA1) was positive for the preictal, the secondary preictal, the tonic, the clonic, and the secondary clonic discharges (92 ± 8.9, 99 ± 2.6, 95 ± 6.3, 98 ± 2.7, and 93 ± 7.1%, respectively). These data strongly argue that SLEs and their individual discharges were generated within the CA3 network.

**Role of GABA in regulating SLE genesis of juvenile slices**

The preceding results clearly indicate that an intense GABA<sub>A</sub> receptor-dependent activity is present at the SLE onset in a region where the discharges are originating from, coincident with high firing probability of pyramidal cells. To explore how these currents are involved in the regulation of SLE recurrence patterns, we applied a selective GABA<sub>A</sub> receptor antagonist, picrotoxin (PTX).

In voltage-clamp experiments with the cells held at 0 mV, PTX blocked outward currents associated with SLE onsets, thus providing pharmacological evidence on the GABAergic nature of these currents (Fig. 7, A and E). The effect was dependent on PTX concentration (1 μM PTX: 74 ± 6%, P < 0.01, n = 3, 10 μM PTX: 7 ± 3%, P < 0.001, n = 4, 100 μM PTX: 0.2 ± 1%, P < 0.001, n = 3; outward current amplitude in percent of control outward currents at the SLE onset; Fig. 7A). PTX significantly decreased inter-SLE intervals, but only higher concentrations were effective (10 μM PTX: 62 ± 7%, n = 5; 100 μM PTX: 45 ± 11%, P < 0.01, n = 5; mean ± SE, in percent of control; Fig. 7, B and E). 10 μM PTX decreased the number of preictal discharges before SLEs (25 ± 16%, P < 0.01, n = 5; mean ± SE, in percent of control) but did not change the duration of SLEs (Fig. 7, C and D). Effects of 100 μM PTX on the number of preictal discharges were not determined because due to the fast recurrence (small inter-SLE interval) the last clonic discharges of an SLE were hard to discriminate from the preictal discharges of the next). After 20 min of washout of either 1 or 10 μM PTX, the amplitude of outward currents were not significantly different from control values, and inter-SLE intervals were not different from values measured in control slices. Interestingly, the number of preictal discharges remained significantly lower than control (1 μM picrotoxin: 30 ± 20%, 10 μM picrotoxin: 44 ± 25%, P < 0.05, n = 5, mean ± SE, in the percent of control).

**Discussion**

By examining the functional dynamics underlying the genesis of spontaneous, recurrent SLEs in the CA3 region of the hippocampal slices from juvenile rats (P10-13), we have found parallel changes in f.p. discharge waveform, HFO, synaptic input dynamics and CA3 pyramidal cell spike timing characteristics during the preictal period. All these changes reflect the gradual build-up of a network state that can be referred to as ictogenesis culminating in the SLE onset. Figure 8 summarizes the most important properties (Fig. 8A) and the development (Fig. 8B) of this network state. Once this network state has been reached, it is maintained throughout the SLE (Fig. 8B) as to most parameters showed similar dynamics during the SLE onset and primary clonic discharges. The most striking feature of the temporal organization of synaptic currents impinging onto single CA3 pyramidal cells at the instant of SLE onset is the advanced arrival of GABAergic input followed by glutamatergic excitation (Fig. 8A, see also Fig. 4). Similar temporal sequence of synaptic inputs has been shown to represent inhibitory mechanism temporarily blocking the spread of epileptiform discharges in the neocortex (Trevelyan et al. 2006). The situation in the hippocampus is somewhat different as this pattern is not associated with SLE spread but ictogenesis instead, most probably because the discharges are generated within the network of hippocampal CA3 under our conditions. The question arises how the CA3 pyramidal cells are recruited into the SLE onset and subsequent discharges once they receive such a strong GABAergic inhibition first. When their intracellular milieu is intact, e.g., recorded with cell-attached pipettes, pyramidal cells show maximal firing precisely at the start of the SLE onset (and clonic discharges) at a time when they receive mostly GABAergic input (Fig. 8A). Intense and synchronous firing of pyramidal cells is also reflected by the strong glutamatergic input that follows the synchronized GABAergic input with a delay consistent with monosynaptic activation. A substrate to such a monosynaptic fast excitation is most likely the abundant recurrent collateral system of the CA3 region (Debanne et al. 1995; Li et al. 1994; Wittner et al. 2007).

This tight temporal association between GABAergic input and CA3 pyramidal cell firing, together with limited coincident glutamatergic drive is paradoxical given that at the developmental stage of the animals used in the present study (P10-13) GABA provides hyperpolarizing/shunting inhibition to CA3 pyramidal cells (Rivera et al. 1999; Tyzio et al. 2007). Our observation that when recorded in whole cell mode, timing of action potentials is critically dependent on the Cl<sup>−</sup> concentration of intracellular solution suggests that indeed functional...
state of GABA<sub>A</sub> receptor-mediated inhibition is critical for the spike timing of pyramidal cells at the start of discharges. As the unperturbed pyramidal cells showed spike timing similar to high-[Cl<sup>-</sup>] loaded cells, we conjecture that GABAergic currents are excitatory and (at least partially) drive the cells to threshold at the start of SLE onset and clonic discharges. However, the fact that GABA<sub>A</sub> receptor blockade by PTX decreases inter-SLE interval indicates that during the time between two SLEs, GABA might provide inhibition just like under control conditions (Rivera et al. 1999; Tyzio et al. 2007). Together these data point to a possible activity-dependent depolarizing shift in the GABA<sub>A</sub> reversal potential during the preictal period. For the early postnatal days, GABA action is depolarizing and remains vulnerable even after P10 (Dhzala and Staley 2003b; Khazipov et al. 2004; Le Van Quyen et al. 2006; Tyzio et al. 2007). Increasing extracellular [K<sup>+</sup>] (to 5 mM in our case) further challenges the systems responsible for the Cl<sup>-</sup>/HCO<sub>3</sub>/Cl<sup>-</sup> homeostasis maintaining hyperpolarizing GABA<sub>A</sub> receptor-mediated currents (Dhzala and Staley 2003b; Korn et al. 1987; Traynelis and Dingledine 1988). Under such circumstances, excessive GABAergic activation may induce activity-dependent rearrangement of Cl<sup>-</sup>/HCO<sub>3</sub> distribution (Fujiwara-
Tsukamoto et al. 2007; Kaila et al. 1997; Lamsa and Taira 2003; Staley et al. 1995), a critical determinant of the functional effect of GABA$_A$ receptor-mediated conductance (Lamsa and Kaila 1997; Rivera et al. 1999; Staley et al. 1995). Our data imply intense GABAergic input to CA3 pyramidal cells during all preictal discharges that may well result in activity-dependent weakening of GABAergic inhibition or eventually emergence of GABAergic excitation during the preictal period. The extent of anion gradient rearrangements is also frequency and synchrony dependent (Lamsa and Taira 2003; Staley et al. 1995). Thus the synchronization of GABAergic inputs during the preictal period may actively support such a process and may represent a vicious circle of icotogenesis. In support of the requirement of functional alterations in GABA$_A$ receptor-mediated effects for SLEs to occur, we demonstrated that PTX shortens inter-SLE interval and reduces the number of preictal discharges. The tight temporal association between GABAergic inputs and CA3 pyramidal cell firing developed gradually during the preictal period. In cell-attached mode, firing only partially overlapped with GABAergic input during preictal discharges but was coincident at SLE onset, tonic, and clonic discharges (Fig. 8B). However, when the intracellular [Cl$^-$] was high, even the small GABAergic input resulted in strong firing (e.g., before the start of early preictal discharges). By contrast, under low intracellular [Cl$^-$] condition, the GABAergic input coincided with suppressed firing even for late SLE stages, such as SLE onset or clonic discharges, and CA3 pyramidal cell spike distribution came closer to the glutamatergic input dynamics (Fig. 8B: tonic and clonic discharges). Role for depolarizing GABA in icotogenesis was suggested by several studies conducted using different seizure models (Cohen et al. 2002; Dzhala and Staley 2003b; 2005; Fujiwara-Tsukamoto et al. 2003, 2007; Khazipov et al. 2004; Köhling et al. 2000; Lopantsev and Avoli 1998; Perez Velazquez 2003), which might be further modulated by differential regulation of spatially segregated GABAergic responses on dendritic and somatic receptors (Cossart et al. 2001; Gullelde and Stuart 2003; Marty and Llano 2005; Romo-Parra et al. 2008). The activity-dependent changes in anion distribution and consequent mutual excitation of interneurons (Fujiwara-Tsukamoto et al. 2007; Lamsa and Taira 2003), together with gap junction coupling (Beierlein et al. 2000; Nyikos et al. 2003; Tamás et al. 2000) might also contribute to synchronization of GABAergic cells.

Hippocampal interneurons, the most probable players of timing are heterogeneous (Freund and Buzsáki 1996; Parra et al. 1998; Somogyi and Klausberger 2005), have differential effects on principal cells (Miles et al. 1996) and diverge in their relation to physiological oscillations (Klausberger et al. 2003; Somogyi and Klausberger 2005) and SLE patterns (Fujiwara-Tsukamoto et al. 2004; Spamparno and Mody 2007; Ziburkus et al. 2006). Although further research on firing patterns of identified interneurons is required to disclose the source of GABAergic inputs described here, one possible candidate is axo-axonic cells, showing a firing pattern consistent with such a role during tetanically induced afterdischarges (Fujiwara-Tsukamoto et al. 2004) and evoking excitatory responses under control conditions in vitro (Szabadi et al. 2006; but see...
The function of GABAergic synaptic input is also outlined by its pronounced reduction during the tonic period, most probably due to action potential inactivation in interneurons (Ziburkus et al. 2006) or reduction of driving force. This may result in a runaway excitation through tonic period (Ziburkus et al. 2006), but as soon as GABAergic input recovers to amplitude comparable to glutamatergic, the pattern of clonoc activity with all its characteristic similarities to the SLE onset returns.

Our results show that at the start of SLE onset and clonic discharges intense firing of CA3 pyramidal cells is not only coincident with a strong GABAergic synaptic input but also with a negative fp deflection-associated short epoch of 400–800 Hz fp HFO (Laszto³zi et al. 2004). The idea of a causal relationship between HFOs (>200 Hz) and seizure genesis is based on spatial and temporal correlation of the two. In a series of studies, Bragin et al. (1999, 2000, 2003) have shown that in vivo oscillatory activity >200 Hz is spatially restricted to the epileptogenic focus and formulated a hypothesis that the emergence of a hyper-synchronous neuronal sub-network drives synchronized inhibition in neocortex. Underlying the gradual development of network synchrony the genesis of epileptiform activity may possibly involve an activity-dependent depolarizing shift of GABA inputs during the preictal phase.

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